Allelopathic effects of *Sphagnum* moss

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Tiivistelmä — Referat — Abstract

Sphagnum moss could be used as a substitute for *Sphagnum* peat as a growing medium. It has the same positive physical properties as peat and it is also a more sustainable option. However, there are some indications that *Sphagnum* moss may have some inhibitory effects on vascular plant seed germination and seedling development.

The aim of this study was to find out whether this is true and due to the low pH of different *Sphagnum* moss species. The tested moss species were *Sphagnum fallax, Sphagnum medium, Sphagnum rubellum* and *Sphagnum* spp. The seed germination on *Sphagnum* moss substrate was tested with lettuce, radish, basil, pine and ryegrass. Also, two additional seed germination experiments were done with lettuce. Seedling growth experiment on *Sphagnum* substrate was tested with lettuce.

The first germination experiments indicated that the dicotyledon species basil, radish and lettuce are sensitive to the allelopathic effect caused by *Sphagnum* moss. In the case of ryegrass and pine no indication of seed germination inhibition was found. The two additional germination experiments confirmed that *Sphagnum* moss and white peat substrates and *Sphagnum* moss and white peat organic matter/water extracts were inhibiting lettuce seed germination. Added lime didn't conclusively explain the inhibition in germination percentages of *Sphagnum* moss substrate when compared to control treatment gauze. Only in the case of radish the raised pH had positive effect on the germination percentage. Therefor it was concluded that the low germination percentage is not explained only by the naturally low pH of *Sphagnum* mosses and *Sphagnum* mosses' other characteristics should be investigated in the future.

In the seedling growth experiment done with lettuce on *Sphagnum medium* growing medium there was no indication of allelopathic effect on seed germination or seedling development. The allelopathic compounds were thought to have been lost in this experiment through leaching when the substrates were watered.

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Allelopathy, *Sphagnum* moss, germination, lettuce, pine, radish, ryegrass, basil, phenolics

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Tiivistelmä — Referat — Abstract
Tutkimus pohjautuu haluun tutkia mahdollisuutta käyttää rahkasammalta (*Sphagnum*) turpeen sijasta kasvintuotannon kasvualustana. Rahkasammal näet omaa samat hyvät fysiologiset ominaisuudet kuin turvekin, mutta sen käyttö on kestävämpi ratkaisu ympäristön kannalta. Esiin on kuitenkin noussut kysymys rahkasammalen sisältämistä allelokemikaaleista, jotka mahdollisesti estävät putkilokasvien siementen itämistä ja häiritsevät nuorten taimien kehitystä.

Tämän tutkimuksen ensisijaisena tavoitteena oli selvittää, haittaako rahkasammal putkilokasvien itämistä. Lisäksi tutkittiin, johtuvatko mahdolliset häiriöt itämisessä ja nuoren taimen kehityksessä vain rahkasammalen luontaisesti alhaisesta pH:sta. Testatut rahkasammallajit olivat *Sphagnum fallax, Sphagnum medium*, *Sphagnum rubellum* ja *Sphagnum* spp. Rahkasammalalustoilla testattiin lehtisalaatin, retiisin, basilikan, männyn ja raiheinän itämistä. Putkilokasveista herkimmäksi osoittautuneen lehtisalaatin itämistä testattiin kahdella lisäkokeella. Myös taimivaiheen kasvatuskoe rahkasammalalustalla toteutettiin lehtisalaatilla.

Ensimmäisen idätyskokeen perusteella kaksisirkkaisten lajien (basilikan, retiisin ja lehtisalaatin) siemenet olivat herkimpiä rahkasammalen allelopaattisille vaikutuksille. Männyn ja raiheinän suhteen ei samanlaista itämisen estoa ollut nähtävissä. Toinen ja kolmas idätyskoe vahvistivat, että lehtisalaatin itäminen estyy voimakkaasti sekä kalkitsemattoman turpeen että rahkasammalen vaikutuksesta. Kalkitus ei poistanut tätä itämisen estoa kokonaisuudessaan minkään testatun lajin kohdalla. Ainoastaan retiisin tapauksessa kalkitus paransi itävyyttä. Tämän vuoksi rahkasammalen itämisen eston syynä ei voi pitää pelkästään matalaa pH:ta, vaan syitä on tulevaisuudessa etsittävä myös rahkasammalen kemiallisista ominaisuuksista.

Taimikasvatuskokeessa, jossa kasvualustana käytettiin *S. mediumia,* ei havaittu allelopaattista vaikutusta lehtisalaatin itämiseen eikä taimen kehitykseen. Tämän katsottiin johtuvan kasteluveteen lisätystä lannoitteesta ja kastelutekniikasta.

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$CaCO_3$	Calsium carbonate
IAA	Auxin
ISTA	International Seed Testing Association
NaPPI	the National Plant Phenotyping Infrastructure
PPFD	Photosynthetic photon flux density

1 INTRODUCTION

With the growing global population, the production of plant-based products for food is critical. In plant production the growing medium or the soil plays a key role in crop cultivation. *Sphagnum* moss peat has been widely used as a growing medium in plant production because of its positive structural and physical properties, low production costs and availability. Unfortunately, peat is a finite or slowly renewable material source (Joosten & Clarke, 2002 p. 25). In a study focusing on *Sphagnum* moss renewability it was found that after harvesting in good conditions the growth of *Sphagnum* moss was renewed by 90 % in seven growing seasons whereas peat forming is a significantly slower process (Pouliot 2015). Harvesting peat also destroys the natural biodiversity of the bogs and decreases the bog carbon storage capacity (Joosten & Clarke 2002 p.33; Gaudig 2014). Therefore, other alternatives for plant production growing mediums should be considered.

Non-decomposed *Sphagnum* fibers have been found to have similar properties to peat and could be used as a replacement for it or as a part of substrate mixture (Caron & Rochefort 2013). Then again, before commercial use of the *Sphagnum* mosses, the special properties of these Bryophytes should be studied. The physiological and chemical properties and the living surroundings of these Bryophytes differ greatly from vascular plants. *Sphagnum* mosses grow on bogs in very compact communities, in wet conditions and with low pH surroundings. These conditions make it difficult or impossible for most vascular plants to survive and prosper. The superiority of *Sphagnum* mosses in bog environments is based on three factors; tightly knit moss communities, the ability to stand very wet or/and dry conditions and the ability to acidify their surroundings. These three competitive factors make it very difficult for vascular plants to thrive in the bog environment.

In addition to these competitive mechanisms there are also indications that some of *Sphagnum* mosses chemical properties, secondary metabolite products also act as an inhibitor when it comes to vascular plant seed germination and seedling development. Up to date relatively little is known about this inhibition effect called allelopathy related to *Sphagnum* moss.

The aim of this study was to find out if *Sphagnum* moss really inhibits some of the vascular plants seed germination and seedling development and if this is only due to its naturally low pH.

2 ALLELOPATHY

Allelopathic effects are seen both in natural and in managed ecosystems. The effects are manifested for example in the diversity, structure, productivity, reproduction, succession and or the establishment of plant communities. Even though allelopathy effects can be seen in the natural plant communities they shouldn't be confused with competition of resources like nutrients, water, light and space between plants (Newman & Rovira 1975. Goldberg 1990. Blum 2011 p.2).

The pioneer of the allelopathy research, Rice (1984), determined the allelopathic effect to be an effect that a plant, including its micro-organisms, has on another plant via chemicals it releases into the environment. These chemical compounds are called allelochemicals, which are mainly plant secondary metabolites or decomposition products of microbes. Allelochemicals can be organic or inorganic compounds, which Rice (1984) divided in to 14 different groups and one miscellaneous group. These groups are 1) simple water-soluble organic acids, straight chain alcohols, aliphatic aldehydes and ketones 2) simple unsaturated lactones 3) long-chain fatty acids and polyacetylenes 4) naphthoquinones, anthraquinones and complex quinones 5) simple phenols, benzoic acid and derivatives 6) cinnamic acid derivatives 7) coumarins 8) flavonoids 9) hydrolysable and condensed tannins 10) terpenoids and steroids 11) amino acids and polypeptides 12) alkaloids and cyanohydrins 13) sulfides and mustard oil glycosides as well as 14) purines and nucleosides. The allelopathic effect can be positive, negative or neutral depending on the allelochemicals potency, and other physiochemical and biotic factors (Blum 2011, p.185).

Allelochemicals are lost from living plants trough passive and active processes (Rice 1984, Bertin et al. 2003, Blum 2014). Passive loss of allelochemicals from the donor plant includes diffusion of low molecular weight compounds from cells (Bertin et al. 2003, Blum 2014, p.7). These low molecule weight compounds are lost from the plant when cells destruct, when the leaves of the plants are shed or as leachates of living, dying or dead tissues such as leaves, stems, roots and flowers get to the surroundings (Bertin et al. 2003, Blum 2011 p.7). Active loss of allelochemicals from donor plant includes secretion and exudation of high molecular weight compounds by cells and the active transport of

organic compounds across cell membranes (Bertin et al. 2003, Blum 2014 p.7). Characterizing of allelochemicals lost from donor plants and understanding the modes of action in receiving plants through field and laboratory bioassays has been the main focus in allelopathy research in the past decades.

Usually, the concentrations of allelopathic compounds are weaker in the soil and plant part mixtures than in intact plants where the concentration of allelochemical compounds is considered to be maximal (Inderjit 1996). Also, allelopathic compounds have complex interactions or co-existing effects with other physiochemical or biotic factors in the soil that should be taken in consideration (Blum 2014, p. 31-35). These other physiochemical and biotic factors could mean/may occur for example in artificial growing media with abnormal micro-organisms which atypically affect allelochemicals (Inderjit 2005). These interfering factors are more present if an allelopathy experiment is done with plant parts, residue or leachates mixed in a growing medium.

In agriculture allelochemicals can potentially be used as growth regulators, herbicides, insecticides, and antimicrobial crop protection products. For this type of use the chemical compounds must be recognized and the concentration potency for wanted reaction as well as the modes of action in the receiving plant have to be known.

When studying allelopathy there are several things that should be taken into consideration when planning a reliable bioassay. Most of the allelopathy research, have been focused on examining the effect of allelochemicals on plant seed germination and seedling development, because the experiments are relatively straight forward and easy to execute. There are two types of approaches that can be taken when studying plant-plant allelopathy on seed germination and seedling development.

The first is the reductionist approach where specific chemical compounds are extracted from a donor plant and their influence on receiving plant’s germination is examined (Blum 2014). This type of research is focused on identifying the allelochemical and on determining the concentration needed to affect seed germination and seedling development in the receiving plants (Belz et al. 2005). Even though this line of allelopathy research has its merits, several researchers have advised to avoid assay comparisons of dose-response curves (Inderjit 2002, Belz et al. 2005), since variation between experiments is often too great and model comparisons become very doubtful which may

result in misleading conclusions. Also, it is not enough to know that a plant has chemical compounds in it in a harmful extent. It is more important to know if these chemical products are naturally excreted into the soil or substrates. The excretions that are forced out of the plant may give a wrong idea of the concentrations of allelochemicals that are naturally excreted from the plant into the surroundings (Blum 2014).

The second way of studying allelopathy is the holistic approach where other physiochemical factors, other than allelochemicals, and biotic aspects are taken into consideration. The other physiochemical factors include pH, soil moisture, nutrients and other inorganic or organic compounds (Blum 2011, p. 35). The biotic factors include for example root and mycorrhizal surface area, soil and plant microbes (Blum 2011, p.31). For example, it is important to take in to consideration the pH as a contributing physiochemical factor for the seed germination and seedling development, because low pH tends to influence negatively most vascular plant species. This gives a more realistic idea of the combination of biochemical factors and allelochemicals influencing the plant cultivation in a real-life situation.

3 *SPHAGNUM* MOSS

There are approximately 150 well known and recognizable *Sphagnum* species, but even as much as 300 species have been described worldwide (Schofield 1985. p. 32). Lately new molecular (DNA sequencing) techniques have given more information about the known species, and made old classifications questionable, like in case of moss *Sphagnum magellanicum* (Brid.) that is now considered to be three different species (Hassel et al. 2018). According to the new taxonomic definition *Sphagnum magellanicum* (Brid.) is only met in South-America and the formelly known *Sphagnum* magellanicum species in the northern hemisphere are devided in to two groups; *Sphagnum divinum* (Flatberg & Hassel) and *Sphagnum medium* (Limpr.) (Laine et al. 2018).

Also, global mapping of *Sphagnum* species has its shortcomings and therefore the number of recognized species has varied considerably. The *Sphagnum* species are found all over the world but the species in the northern and temperate regions are perhaps better charted than the species on the southern hemisphere (Gajewski et al. 2001, Whinam et al. 2003, Seneca 2008). In these two areas the species diversity has been estimated to be greater in temperate than in boreal regions (Seneca 2008, Shaw et al. 2010). Then again, largest

areas covered *Sphagnum* mosses are found from in the boreal regions (Clymo 1982, p. 236). For instance, Koponen et al. (1977) identified 69 *Sphagnum* species and subspecies just in Finland. *Sphagnum* species can be taxonomically divided in to sections based on their growing habitat and morphological properties (Crum 1984, Flatberg 2002).

In Finland, the southern boreal regions are categorized by raised bogs and the northern boreal regions are categorized by aapa mires and in the most northern parts by palsa mires (Pakarinen 1995). Raised bogs are ombrotrophic mires that are mostly formed by hummocks and the vegetation rely on rainwater for its water supply. The *Sphagnum* species common in raised bogs are *Shagnum magellanicum* (Brid), *Sphagnum angustifolium* (Russ.) C. Jens, *Sphagnum russowii* (Warnst.) and *Sphagnum fuscum* (Schimp.) (Pakarinen 1995. Seppä 2002). Aapa mires are minerotrophic open fen environments that rely on water and nutrient supply from the surrounding areas (Pakarinen 1995. Seppä 2002). The most common *Sphagnum* species in the aapa mire is *Sphagnum papillosum* (Lindb.) (Seppä 2002). The typical *Sphagnum* species for the wet and permafrost cored palsa mire is *Sphagnum lindbergii* (Schimp. ex Lindb.) (Seppä 2002).

3.1 *Sphagnum* moss and competition

Sphagnum mosses form dense and tight lawns in the hollows or carpet like hummocks in the mire environment. Different *Sphagnum* species are specialized in different combinations of water level, pH and shading. Forming diverse and dense communities is the principal way they compete against vascular plant invasion.

The water conditions can vary significantly between different mire types. The nutrients available in these mires are also sparse do to this fact. The ability to withstand low nutrient conditions is the second reason that makes *Sphagnum* mosses good competitors in the mire environment. These low nutrient mires, raised bogs, are the environments in Finland where most of the peat moss is harvested.

The third way *Sphagnum* moss makes mire environment difficult place for other species to thrive is its ability to acidify its surroundings down to pH ≤4 (Vitt 1983). This is due to the *Sphagnum* mosses a high cation exchange capacity that at the same time acidifies the environment and traps nutrients (Verhoeven 1997). When Vitt (1983) studied four

oligotrophic basin mires in northwestern Ontario he found out that the nutrient amounts, conductivity and pH varied strongly (4,0-5.7) between the mires, and there were differences even between interior and edge portions of the same mire.

Sphagnum mosses are not grazed by animals and that gives this species a competitive edge against vascular plant species. The last competitive factor favoring *Sphagnum* mosses existence in the bog environment is their special physical and chemical properties that make *Sphagnum* mosses very decay resistant (Verhoeven & Liefveld 1997).

3.2 Physiological properties of *Sphagnum* moss

Sphagnum mosses are part of phylum Bryophyte group of mosses, class Sphagnopsida (peat moss) that represent non-vascular plants (Shaw et al. 2016). Unlike vascular plants *Sphagnum* mosses do not have lignin in their cell walls nor do they have roots, and they do not produce flowers and seeds but reproduce through spores.

Sphagnum mosses have one vertical stem and at the top of the stem there is an apical meristem surrounded with many small branches of limited growth that are tightly crowded. They form the capitulum, from where the plant grows upwards (Håkan & Jeglum 2006, Figure 1). *Sphagnum* mosses form lateral branches along the stem in regular intervals (Håkan & Jeglum 2006, Figure 2). These lateral branches have leaves attached to them. In *Sphagnum* mosses there are also leaves that are growing directly from the stem. *Sphagnum* moss leaves are formed by one cell thick layer of hyaline cells, and between them there are enclosed or chlorophyllose cells (Clymo 1982 p. 229). About 90% of the *Sphagnum* moss cells are dead, long, empty, thin walled but surprisingly strong structured hyaline cells (Clymo 1982, p. 229). The *Sphagnum* mosses regulate water flow by storing water in to hyaline cells (Weston et al. 2015). From the water table, water gets to the cells through capillary flow along the exterior of the stems, branches and capitula (Proctor 1982, Figure 2). *Sphagnum* mosses also store rain water directly from rainwater and can hold large amounts of water, even 20 times their dry weight (Proctor 1982).

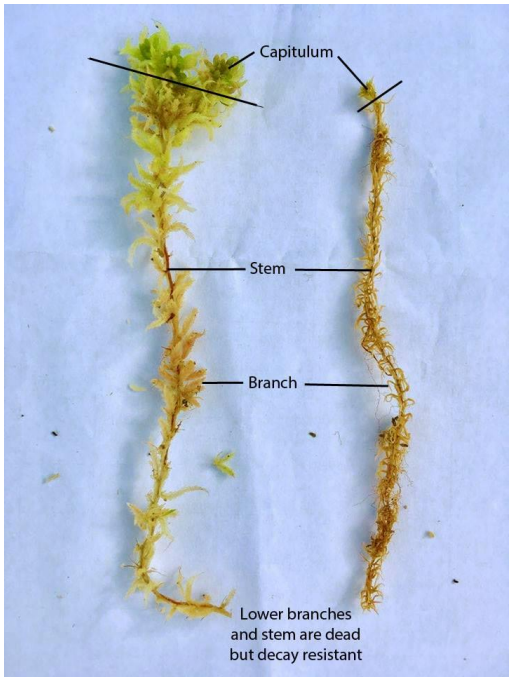


Figure 1. Morphological structure of two *Sphagnum* moss species. Left: *S. medium* and right: *S. fuscum*.

The special property of *Sphagnum* mosses is that the upper part of the stem continues to grow while the lower part of the stem dies and starts to decompose into peat (Clymo 1982, p. 231). Thus, *Sphagnum* moss peat is morphologically characterized by two distinct layers; the top living part (moss) that is less than 10 cm thick and the decaying layer below it, where the decay leads to the accumulation of a peat layer that can be many meters thick (Chiapusio et al. 2013). The slow decomposition of the dead parts is partly due to the nutrient conditions and anaerobic, and acidic conditions prevailing in the bog environment (Bragazza 2007). The micro-oranisms present in the fen versus bog differ and so does the decomposition rate (Bragazza 2007). The slow decay is also caused by the the species-specific chemical properties of *Sphagnum* mosses, but so far it is unclear which biochemicals are responsible of this decay resistance (Verhoeven & Liefveld 1997).

3.3 Chemical properties of *Sphagnum* moss

Sphagnum mosses are known to produce secondary metabolite products that seem to have no obvious role in plant metabolism, but work as a defence mechanism for the plant (Baas 1989, Verhoeven & Liefveld 1997). In addition to allelopathic effect against invasive

vascular plant species the most obvious reasons for the production of these secondary metabolite products is resistance against pathogens and herbivores (Wink 1988).

In *Sphagnum* moss the production of secondary metabolite product is promoted by biotic and abiotic stress (Baas 1989; Lambers & Poorter 1992). Secondary metabolite products accumulate to *Sphagnum* mosses due to restricted water and nutrient supply (Lambers 1993). Bragazza and Freeman (2007) demonstrated that low nitrogen availability increased polyphenol content in the *Sphagnum* moss litter. This led them to conclude that in *Sphagnum* mosses proteins and polyphenols compete for the same resource, nitrogen (Bragazza & Freeman 2007). It has also been shown that low nitrogen availability in vascular plants leads them to use carbon for secondary metabolite production (Bryat et al. 1983). The roles of the secondary metabolite products such as polyphenols have also been studied with the focus on their potential allelopathic effect (Blum 1996, Inderjit 1996).

Sphagnum mosses produce two types of organic secondary metabolites; carbohydrates and phenol compounds. Understanding the function of the compounds could be the key factor in understanding the putative allelopathic effect produced by the *Sphagnum* mosses.

3.3.1 Carbohydrates

Carbohydrates are the first big group of secondary metabolite products in *Sphagnum* moss and they consist of three functional groups carbonyl, carboxyl and hydroxyl (Taskila 2016). The biggest portion, 10 – 30 % of *Sphagnum* mosses dry weight consists of uronic acids that are sugar acids with both carbonyl and carboxylic acid functional groups (Clymo & Hayward 1982). The exchange of the carboxyl group protons is the basis of the well-known good ion exchange capacity of *Sphagnum* that also acidifies the bog environment (Verhoeven & Liefveld 1997). For example, the ions that *Sphagnum* needs for growth, like Mg^{2+} and Ca^{2+}, are transported through proton pumps while at the same time *Sphagnum* releases hydrogen ions H^{+} to the environment.

Sphagnum moss also polymerises uronic acids in to glycuronoglycans that structurally resemble complex pectins of higher plants but differ from them in containing 25 % ketouronic acids and highly reactive carbonyl groups (Painter 1998). These sugars

comprise 60 % of holocellulose in hyaline cell walls of the *Sphagnum* mosses (Painter 1998).

3.3.2 Phenolics

Phenolics or polyphenols are the second big group of secondary metabolite products and they consist of large variety of molecules. *Sphagnum* acid [p-hydroxy-beta-(carboxymethyl)-cinnamic acid] is the most abundant phenolic substance isolated from *Sphagnum* spp. Rasmussen et. al (1995) excreted, identified and measured the amount of some of these phenol compounds from two *Sphagnum* species *S. fallax* and *S. magellanicum* (Table 1). They concluded that most of the *sphagnum* acid was in buffer-soluble form and not bound to the cell wall, whereas p-hydroxyacetophenone, hydroxybutenolide, *p*-hydroxybenzoic acid, *p*-Coumaric acid and *trans*-Cinnamic acid were primarily bound to the cell wall (Rasmussen et al. 1995).

Table 1. Phenolic acid amounts and their proportion of all buffer-soluble phenolics in two *Sphagnum* moss species (Rasmussen et al. 1995).

Phenolic compound	*Sphagnum magellanicum*	*Sphagnum fallax*
***Sphagnum* acid**		
Total amount μmol g^{-1} dryweight	2,3	2,9
Buffer soluble amount %	58	61
***p*-Hydroxyacetophenone**		
Total amount μmol g^{-1} dryweight	1,6	1,5
Buffer soluble amount %	6	6
Hydroxybutenolide		
Total amount μmol g^{-1} dryweight	1,4	1,3
Buffer soluble amount %	3	2
***p*-Hydrobenzoic acid**		
Total amount μmol g^{-1} dryweight	0.8	0.9
Buffer soluble amount %	2	5
***p*-Couramic acid**		
Total amount μmol g^{-1} dryweight	0.3	0.3
Buffer soluble amount %	6	7

While studying phenolic constituents of living *Sphagnum* moss Rasmussen et al. (1995) noticed that leaching and excretion of *sphagnum* acid, *sphagnum* acid ethyl ester, hydroxybutenolide, p-hydroxybenzoic acid, *p*-Coumaric acid and *trans*-Cinnamic acid was enhanced when *S. fallax* was cultivated in a bioreactor. In their study Rasmussen et al. (1995) used both *Sphagnum* moss samples collected from a natural growing habitat (*S. magellanicum* and *S. fallax*) and *Sphagnum* moss samples axenically cultivated in continuous feed bioreactors (*S. fallax* and *S. cuspidatum*). The amount of buffer soluble phenolics was measured from the bioreactors' effluent media (Rasmussen et al. 1995).

Castells et al. (2005) studied *Sphagnum* spp. leachates and their effect on white spruce (*Picea glauca (*Moench*)* Voss) germination. No significant release of phenolic compounds was found in their study, even though Rasmussen et al. (1995) reported that in *Sphagnum* mosses, most of the phenols are in free water-soluble form and can be excreted into the bog water around the mosses. The amount of phenol compounds is known to vary seasonally in *Sphagnum* mosses. Phenol content can differ from one shoot part to another; the capitulum held the largest amounts of phenols that are found in the *Sphagnum* moss studied by Jassey et al. (2011).

The modes of action of secondary metabolite products and especially phenolics in suppressing seed germination and seedling development have been of great interest in the allelopathy studies. The actions of carbohydrates and phenolics that also exist in *Sphagnum* mosses have been a great interest in allelopathy studies.

3.3.3 Modes of action of Sphagnum secondary metabolites

Uronic acids (galacturonic acid and 5-keto-D-mannuronic acid) of *Sphagnum* moss are responsible of the acidifying effect that is seen in the bog environment water table. Uronic acids become ionized in pH above 2 and create new cation exchange sites, which leads to exchange of hydrogen ions for nutrient cations in the bog water table (Clymo 1963, Rydin & Jeglum 2006). On the other hand, phenolics ionize in alkaline pH > 7 and therefore do not contribute to acidifying trough the ion exchance in the bog environment where the pH is usually much lower (Clymo 1963).

In the case of phenolic acids, the membrane uptake of ferulic and p-hydroxybenzoic acid, has been found to be concentration and pH dependent when studied with *Cucumis sativus*

(Shann & Blum 1987). With a lower pH and higher external consentration the transfer into and across the membrane is enhanced (Shann & Blum 1987). Einhellig (1995) proposed membrane disturbances as the mode of action of these phenolic acids. He suggested, that after their entry through the membrane, phenolic acids may cause depolarization of the cell membrane that causes nonspecific efflux of both cations and anions accompanying increased cell membrane permability (Einhellig 1995). These disturbances lead to changes in ion influx and retention with an inhibition of ion uptake (Einhellig 1995). In soybean roots cinnamic and benzoic acids have been suggested to be additional causes for structural changes in membranes including in a variety of membrane proteins (Baziramakenga et al. 1995).

In his study Blum (1996) concluded that some simple phenolic acids like *p*-hydroxybenzoic, *p*-Coumaric and ferulic acids can cause a broad range of phytotoxic damage in a vascular plant, but that they all have the same apparent mode of action. These phenolic compounds inhibit hydraulic conductivity and nutrient uptake by roots of the plants, resulting in growth inhibition (Blum 1996).

It has also been hyphothesised that phenolic compounds interact with a plant hormone auxin (IAA) inducing growth which leads to misshapen, short and stubby roots (Tomaszewski 1966, Einfellig 2005). Then again, these results are not conclusive on behalf of inhibition or activation of IAA. There are also scattered reports of phenolic acids ability to influence on a variety of enzymes produced by plants if they are sufficient in concentration and located at the site of enzymatic functions (Leslie 1988, Devi & Prasad 1992). At the early seedling stage plants are very active in hormone-mediated growth responses and these responses, are easily influenced by phenolic acids (Einhellig 2005).

In a study done by Blum (1996) it was found that amounts of individual phenolic compounds recovered from field soils were very low. In fact, the natural amounts measured from the no-till soil were so low that they couldn’t be responsible for similar seed and growth inhibition executed in in vitro conditions (Blum 1996). Therefore, it has been suggested that the joint action of several phenolic acids could explain allelopathic growth inhibition patterns seen in field and laboratory experiments (Einhellig 1995. Blum 1996).

3.4 *Sphagnum* moss and allelopathy

There are some indications that some vascular plants might be sensitive to the allelochemicals/secondary metabolites produced by *Sphagnum* moss. In nature, the positive effect on seed germination caused by mosses, can usually be explained by good moisture conditions, which is a biotic factor (Zamfir 2003). The negative effect that *Sphagnum* moss has/ may have on vascular plant seed germination in its natural bog environment has been contributed to the low light intensity inside the tightly knit moss carpet and the drier microhabitat of the raised bog (biotic factors) and allelopathy (Zamfir 2003).

Chiapulsio et al. (2013) performed an experiment where both *Sphagnum magellanicum* and *Sphagnum fallax* had an inhibitory effect on *Loliun perenne* and *Pinus uncinata* seed germination but had no effect on *Raphanus sativus* seed germination. It was speculated that *Sphagnum* mosses produce allelochemicals such as phenol compounds and *sphagnum* acid that are harmful for vascular plants seed germination and growth (Chiapulsio et al. 2013).

Oberpaur et al. (2010) tested *S. magellanicum* as a part of a substrate mixture where 60 % moss was combined with 40 % humus or compost. This mixture was found to be suitable for lettuce growing. Also, Michel et al. (2011) concluded in their study that *Sphagnum australe* did not exhibit inhibition on *Lactuca sativa* seed germination and at low water extract concentrations (1 %) it had a stimulatory effect on radicle growth. In his study Hytönen (1992) concluded that *Sphagnum cuspidatum* did not inhibit *Pinus sylvestris* germination.

However, in many cases when the seed germination experiment is done with *Sphagnum* water extracts the results have been difficult to interpret or they have been counterintuitive (Whitehead et al. 2018). With mixed study results and variety of methods used an uncertainty remains from the previous study results. There is a real need to find out what kind of allelopathic effect *Sphagnum* mosses have on vascular plants; negative, positive or neutral and if these are due to biotic factors or special chemical properties. It is important to find out if allelopathic effects are also shown in normal growing conditions of the most common vegetable and ornamental plants because this is where *Sphagnum* moss is most likely used as a substrate in the future.

4 AIMS OF THE STUDY

The main aim of this study was to find out if *Sphagnum* moss is exhibiting inhibition on vascular plant seed germination when used as a growing medium. The hypothesis was that this inhibition is caused by *Sphagnum* mosses allelochemicals. The other aims of the study were to investigate if 1) if there are differences between monocotyledon, diocotyledon and gymnospermous seed germination on *Sphagnum* moss growing medium 2) if the seed germination inhibition is caused only by the naturally low pH of *Sphagnum* moss growing medium 3) if differences in seed germination inhibition depend on *Sphagnum* moss species or on the length of moss's storage time and 4) if in the case of seed germination inhibition also the seedling development is influenced.

5 MATERIALS AND METHODS

The *Sphagnum* moss allelopathic effects were studied in three different germination experiments and one seedling growth experiment in NaPPi growth system. All these experiments were done in in the University of Helsinki facilities in Viikki, Finland. The first germination experiment was done in the summer 2016, the second germination experiment was done in October 2016 and the third germination experiment and seedling growth experiment were done from May to April in 2017.

5.1 *Sphagnum* moss

From the *Sphagnum* moss samples *Sphagnum* spp. was collected in September 2013 from Neva-Lyly, Karvia (62°19.13'N, 22°84.26'E). The moss consisted of *Sphagnum fuscum* ((Schimp.) H. Klinggr.) (50 %), *Sphagnum medium* (20 %), *Sphagnum papillosum* (Lindb.) (10 %), *Sphagnum balticum* ((Russow) C. E O. Jensen)) (10 %) and *Sphagnum rubellum* (Wilson) (10 %). The moss was dried on greenhouse tables, cut to 30 cm pieces and stored in plastic cases in greenhouse corridors. This moss was included to the study to investigate the effects of storage time on the allelopathic effects.

Additional moss samples including *Sphagnum fallax* ((H. Klinggr.) H. Klinggr.), *Sphagnum medium* and *Sphagnum rubellum* were collected in May 2016 from Kaljakankaansuo, Vahojärvi, Parkano. The samples were stored in black plastic bags in a cold storage at +1 C until use. The mosses were identified with the help of Harri Vasander, the professor of bog ecology at the University of Helsinki.

Fibers of each three *Sphagnum* species were separated manually from other moss species and vascular plant species and then dried in a heated cupboard at the temperature of 30-32 °C for 24 hours. The temperature was kept low to ensure it wouldn't excessively modify the chemistry of compounds and that the micro -organisms present in the *S.* moss would not die. After drying, the mosses were sliced with scissors into 5 - 15 mm pieces to simulate woodchipper treated *Sphagnum* moss product in the real-life situation (Figure 2 & 3). The cutting also ensured that the moss fibers cell structure was broken.

Figure 2. Manually separated and dried *S. medium* fiber before (left) and after (right) cutting in to 5-15 mm lengths.

Figure 3. May 2016 harvested *Sphagnum* species used as a growing medium in the experiments. From left to right *S. fallax, S. rubellum* and *S. medium* and 2013 collected *Sphagnum* spp.

5.2 Plant material/ Test plants

Five commonly grown vascular plant species were chosen to the experiment. Lettuce *Lactuca sativa* 'Australische Gele' and 'Grand Rapids', ryegrass *Lolium*, radish *Raphanus sativus var. sativus* 'Cherry belle', basil *Ocium basilicum* 'Mariam' and pine *Pinus sylvestris* were chosen for the seed germination experiment. The seeds were packed by Schetelig Oy Finland except for pinus sylvestris which was packed by Siemen Forelia Oy, Rovaniemi, Finland.

Part of the selected plant species (lettuce, radish, ryegrass) had in the previous studies shown sensitivity to the *Sphagnum* mosses allelochemicals or other known organic compounds *Sphagnum* mosses produce, produced by some other plant species. On the other hand, also species (basil and pine) that have not shown sensitivity to *Sphagnum* moss allelochemicals were chosen to the experiment. The selection was also based on having species with different morphological traits; monocotyledons (ryegrass), dicotyledons (lettuce, radish and basil) and gymnosperms (pine).

5.3 Prerаration of treatments experimental conditions and experimental design

5.3.1 Preparations for germination experiments I and II

From every moss species, *S. fallax*, *S. rubellum*, *S. medium* and *S.* spp. there was a treatment with and without $CaCO_3$ (Calcium carbonate, 99%, extra pure. Acros Organics. Lot: A0369056). First 4g of dried moss (a 10 mm thick layer) was measured on a petri dish and then distilled water was added (Figure 4). The amount of water was 60 ml for *S. fallax*, *S. rubellum* and *S. spp.* and 70 ml for *S. medium*. The amount of water needed to reach the saturation point was tested by adding 100 ml of water to 4 g of dried moss on a petri dish. In the second germination experiment in the case of white peat, 4 g of peat, 30 ml of water and 110 mg of $CaCO_3$ (or no $CaCO_3$) was used in this experiment. The water amount was lower than with the *S.* mosses because the white peat was not dried and therefore had more moisture in it than the *S.* mosses. After 24 hours the non-absorbed water was poured out and measured. The amount of non-absorbed water was subtracted from the 100 ml. The moss-water mixture was smoothed out by pressing with a measuring cup.

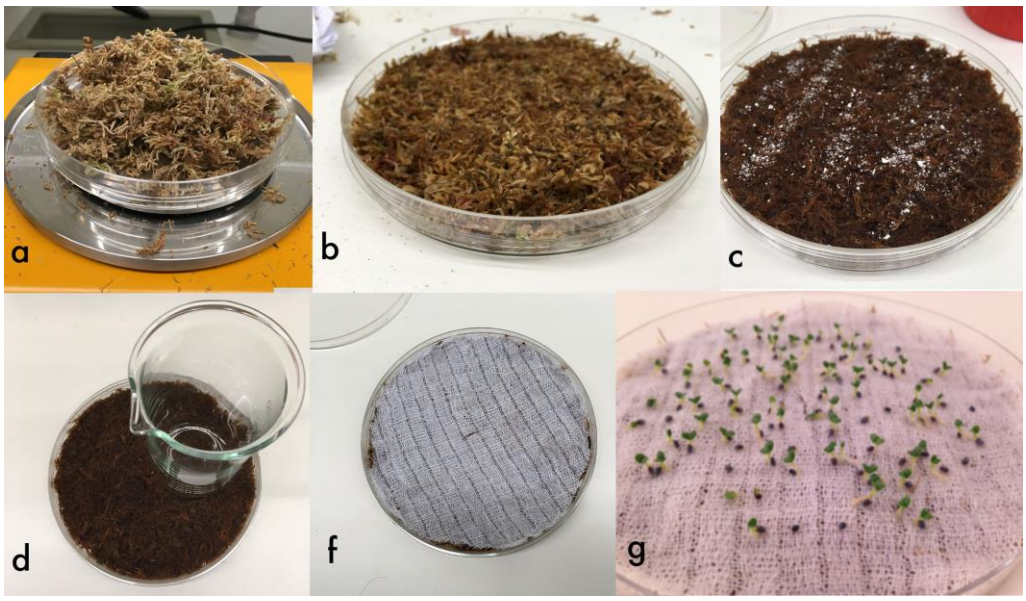


Figure 4. Execution of basil seed germination experiment.
a) measuring dry *S. medium* on a petridish b) *S. medium* after added water and smoothing over c) $CaCO_3$ scattered on top of *S. rubellum* d) pressing the moss water $CaCO_3$ mixture f) adding gauze on top of the growing medium and g) seeds germinating on top of the gauze.

With the *S.* mosses treated with lime, the $CaCO_3$ (*S. fallax* 110 mg, *S. rubellum* 130 mg, *S. medium* 110 mg and *S. spp.* 130 mg) was scattered evenly on top of the water saturated moss and pressed with a measuring cup to ensure $CaCO_3$ dissolving evenly to the water (Figure 4). With *P. sylvestris* germination experiments the amount of added $CaCO_3$ was 10 mg less than in other experiments (*S. fallax* 100 mg, *S. rubellum* 120 mg, *S. medium* 100 mg and *S. spp.* 120 mg, Figure 4). $CaCO_3$ dissolves only in acidic pH conditions, therefor the $CaCO_3$ was added 24 hours after the water was added. The amounts of lime were determined in pre-trials to achieve the pH of 5.5-6.5 (lettuce, ryegrass, radish and basil) and 4.5-5.5 (pine).

The control treatments in these experiments were Whatman 1 filter paper (Cat No 1001-150. Darmstadt, Germany) in two layers and 100 % cotton gauze (Eurokangas, Finland), that both had the optimal pH of 5.8 - 6.0. The control dishes were watered with 9 ml distilled water. The double filter paper was chosen to the experiments as a control because it is commonly used in seed germination experiments. The gauze was chosen to the experiments as a control because it was used in all *Sphagnum* treatments between the moss fibers and the tested seeds. The gauze controls were targeted to check that the gauze was not causing any abnormality in the experiments per se.

Gauze was added on top of the moss to avoid direct contact with the seeds and the moss (Figure 4). A 10 mm cut was done in the middle of the gauze to enable the watering of the growing medium with a pipette during the experiment (Figure 4). One hundred seeds were sown on one 150 mm petri dish (Sarstedt, N*ü*mbrecht, Germany, Figure 4).

The seeds were germinated in a growth chamber with a temperature 20 – 22 °C, photoperiod of 16 hours and average PPFD of 140 $\mu molm^{-2}s^{-}$ (n=8). One treatment had four replicates, 400 seeds altogether. The experimental design was completely randomized but the petri dishes were not moved during the experiment because of the stable temperature and the uniform light conditions.

5.3.2 Preparations for germination experiment III

In the third germination experiment, moss-water extracts were used instead of solid moss. The treatments included *S. rubellum*, *S. medium*, *S.* spp. and white peat, all with and without liming. The moss samples were treated similarly as in the previous germination experiments I and II. The amount of dried moss and white peat was 8 g per experimental unit, double the amount when compared to the two previous experiments. The added $CaCO_3$ amounts were 230 mg for *S. rubellum*, 220 mg for *S. medium*, 260 mg for *S.* spp. and 220 mg for white peat, double the amount when compared to the two previous experiments. 24 hours after adding the lime the water was squeezed out of the moss with a press and filtered through gauze. For every treatment 9 ml of moss-water extract was added on a petri dish lined with double filter paper. The control was a double paper with 9 ml distilled water.

The experimental conditions and experimental design remained the same as in the two previous germination experiments. The seeds were germinated in a growth chamber with a temperature 20 – 22 °C, photoperiod of 16 hours and average PPFD of 140 $\mu molm^{-2}s^{-}$ (n=8). One treatment had four replicates, 400 seeds altogether. The experimental design was completely randomized but the petri dishes were not moved during the experiment because of the stable temperature and the uniform light conditions.

5.3.3 Preparations for seedling growth experiment

The treatments applied were dried *S. medium*, commercial peat-based substrate for lettuce growing (White 620. Kekkilä pH 6.0) and white peat without added lime or fertilizers (Luonnonturve, Kekkilä pH < 4.0. von Post scale H1-H3). The pot size was 200 ml and the amount of dried *S. medium* was 8 g per pot, commercial peat (not dried) 45 g per pot and white peat (not dried) 60 g per pot. On top of the substrates the lime (Puutarhurin kalkki, Nordkalk) was added as follows; *S. medium* 2.5 g, white peat 6 g and commercial peat (no added lime). Water was then added to the substrates from above as follows; *S. medium* 140 ml, white peat 120 ml and commercial peat 100 ml. The fertilizer (Vihannes-Superex, NPK 9-5-31. Kekkilä) was added to the water (11 g fertilizer/ 10 l water) for *S. medium* and white peat substrate. The electric conductivity (EC) of this mixture was 1,4. The targeted pH was 5.5 - 6.5 and targeted EC 1.0. The pH and EC of all the substrates was measured to ensure equal growing conditions. The pH varied between 6.1 - 6.2 (n=9) and EC varied between 0.8 - 1.3 (n=9). Three seeds were sown into each pot to ensure that all the pots had one viable seedling.

The seeds were sown on 24.3.2017 and the seed were germinated in a growth chamber with a night temperature of 16.5 °C and day temperature of 17.5 °C, photoperiod of 12 hours and the mean Photosynthetic Photo Flux Density of 206 $\mu molm^{-2}s^{-}$ (n=10). The extra seedlings were removed with tweezers on 27.3.2017 and the plant trays were moved into NaPPI growth chamber. In the NaPPI growth chamber the temperature was fixed to 20 - 22 °C and the PPFD on a storage shelf was 140 $\mu molm^{-2}s^{-}$. The pots were watered every other day with 20 ml of water. All the three treatments had five plants and two replications.

5.3.4 PH conditions

The $CaCO_3$ was added to ensure the optimal pH (5.5 – 6.5) for the vascular plant germination with the exception of pine that requires lower pH 4.5-5.5. In the preliminary experiments the added lime caused the *S.* mosses pH to have great variability. Therefore, measuring the pH was continued throughout the experiments and the pH measurements were done both before (24 hours after adding the $CaCO_3$) and after the experiments when possible (lettuce, ryegrass, pine) (Table 2). Because a stable pH couldn't be achieved, it

was accepted that the organic substrates with added $CaCO_3$ had the pH between 5.0 and 7.0. Before and after experiment measurement of pH was done in lettuce seed germination experiment in the case of *Sphagnum* mosses without added $CaCO_3$ (Table 3). The pH measurement was done from water extract (50 ml) squeezed from the growing medium. After measurement the water extract was poured back on the moss fibers on the petri dish.

Table 2. Mean pH values measured before and after the ryegrass, pine and lettuce seed germination experiments of *Sphagnum* moss substrates with added $CaCO_3$. *L. multiflorum* and *L. sativa*: *S. fallax* and *S. medium* 130 mg; *S. rubellum* and *S.* spp. 110mg. *Pinus sylvestris*: *S. fallax* and *S. medium* 100 mg; *S. rubellum* and *S. spp*. 110mg +/- Standard deviation shown in the table (n=4).

	L. multiflorum		*P. sylvestris*		*L. sativa*	
	Measured mean pH					
	before	after	before	after	before	after
S. fallax	6.5 (+/-0.4)	6.1 (+/-0.2)	6.9 (+/-0.1)	6.0 (+/-0.1)	7.0 (+/-0.6)	6.4 (+/-0.1)
S. rubellum	5.0 (+/-0.1)	5.2 (+/-0.1)	5.8 (+/-0.6)	5.3 (+/-0.3)	6.5 (+/-0.5)	5.5 (+/-0.1)
S. medium	5.6 (+/-0.3)	5.5 (+/-0.1)	5.6 (+/-0.3)	5.3 (+/-0.3)	6.5 (+/-0.3)	5.8 (+/-0.3)
S. spp.	5.3 (+/-0.7)	5.0 (+/-0.1)	5.2 (+/-0.6)	4.9 (+/-0.3)	6.6 (+/-0.1)	5.1 (+/-0.3

Table 3. Mean pH values measured before and after the lettuce seed germination experiments of *Sphagnum* moss substrates without added $CaCO_3$ +/- Standard deviation shown in the table (n=4).

	L. sativa	
	before	after
S. fallax	4.0 (+/-0.03)	4.5 (+/-0.002)
S. rubellum	3.9 (+/-0.05)	4.0 (+/-0.03)
S. medium	4.1 (+/-0.02)	4.2 (+/-0.02)
S. spp.	3.5 (+/-0.02)	3.7 (+/-0.04)

5.5 Germination experiment I

The objective of this experiment was to study vascular plant seed germination on a general level to find out if some of the species were more susceptible for the putative allelopathic effects of *S.* mosses when used as a growing medium. The tested vascular plant seeds were basil, ryegrass, radish, pine and lettuce. The used growing mediums were four *S.* moss species *S. fallax*, *S. rubellum, S. medium* and *S.* spp. All these *S.* moss growing mediums had two groups, one without added $CaCO_3$ and one with added $CaCO_3$,

producing pH levels below 5.0 and over 5.0. The germinated seeds were counted according to the ISTA (International Seed Testing Association) rules with added count days with the longer germination experiments (Table 4).

Table 4. Counting days in the seed germination biassays. *L. sativa* seeds germination was counted two times, the first and the last day of counts. The other species had two additional count days between the first and the last count days. The results are shown according to the last day of count if not mentioned otherwise.

Species	1st day of count	2nd day of count	3rd day of count	Last day of count
O. basilicum 'Mariam'	4	7	10	14
L. multiflorum	5	8	10	14
R. sativus var. sativus 'Cherry belle'	4			14
P. sylvestris	7	11	14	21
L. sativa 'Australische gele'	4	-	-	7

5.6 Germination experiment II

The objective of this experiment was to further study lettuce seed germination on the *S.* moss growing mediums. Second experiment was done with *L. sativa* 'Australische gele' seeds on *S. medium* and *S.* spp. growing mediums and on white peat (Lunnonturve, Kekkilä. pH < 4,0) growing mediums with and without added $CaCO_3$, producing pH levels below 5.0 and over 5.0. These *S.* moss mediums were chosen to the second experiment because they had the lowest germination percentages in the first experiment. The white peat was chosen to the experiment to be an organic control group with the same pH level and because it has similar physical properties (water holding capacity) as the *S.* mosses, which the control group double filter paper was thought to be lacking. This experiment differed from the previous lettuce seed germination experiment by having white peat added to the experiment. The seeds were counted from the second day on, twice a day.

5.7 Germination experiment III

The objective of this experiment was to again further study lettuce seed germination on the *S.* moss growing mediums. To exclude the potential physical exxects that moss and peat fibres may have on germination, the experiment was done with press water extracts from *S. rubellum*, *S. medium*, *S.* spp. and white peat. Before the pressing, the samples were treated the same way as in two previous experiments only the amount of water changed and was 140 ml with all the *S.* moss species and white peat. This was due to practical reasons, to be able to get sufficient amount (9 ml) of water extract from moss samples. It was noticed in the preliminary tests that the smaller sized *S.* moss species (*S. fallax*, *S. rubellum*, *S. spp*.) and white peat were able hold the added water more tightly in their surface area and cell structure than the bigger *S.* moss species *S. medium*. The press water extract was squeezed out of moss water mixture, wrapped in gauze, with a potato press. Double filter paper was watered with 9 ml of this press water extract and the control was 9 ml distilled water on double filter paper. The seeds were counted from the second day on, twice a day.

5.8 Seedling growth experiment with *Lactuca sativa*

The seeds used were L. sativa ‘Australische gele’ and L. sativa ‘Grand rapids’. All the study groups had ten replicates. Randomized block design couldn’t be done in the NaPPi growth chamber because the tray lines had to consist of the same treatment when photographed automatically. Therefore, there were four trays with five pots of each substrate and two of these trays with lettuce ‘Australische gele’ and two these trays with lettuce ‘Grand rapids’.

5.4 Observations on seed germination and seedling development

The seed germination was counted following International Seed Testing Association rules for seed germination testing (ISTA 2019) which gives guidelines of the environment and the time needed to test different plant species. The seed was considered germinated when it had at least one millimeter of radicula growth and the hypocotyle was turning green due to photosynthesis. After each count the substrates were watered with 5 ml of distilled water if there was no condenced moisture seen at the bottom of the petri dish. The germinated seeds were removed with tweezers from the petri dish after counting. The

length of the radicula was not measured like usually in the seed germination experiments, because the removal of the developing seedling from the *S.* moss substrate sometimes ended up breaking the radicula. Visual observations of the radicula growth were made.

In the National Plant Phenotyping Infrastructure NaPPI growth chamber the seedling development was measured and photographed daily. The key focus was on the seedling growth (RGB imaging) and development. After two weeks in the NaPPI growth chamber the fresh weight and dry weight were measured and leafs per plant were counted.

5.9 Statistical analysis

Data was analyzed using SPSS (SPSS 24.0. IBM SPSS Statistics, Armonk, NY, USA). All the germination data was arcsine transformed before statistical analysis to ensure homogeneity of variance (Badger and Ungar, 1989). Arcsine transformation is commonly used for seed germination percentage data in research (Baskin and Baskin 2014). Arcsine transformation was done in Spss with the following computation:

$$\text{Germination \%} = \frac{\text{Arsin(Sqrt(germinated seeds/whole amount of seeds))*45}}{\text{Artan(1)}}$$

A two-way ANOVA was conducted to examine the effects of pH and substrate levels on germination percentage. Residual analysis was performed to test for the assumptions of the two-way ANOVA. Outliers were assessed by inspection of a boxplot, normality was assessed using Shapiro-Wilk's normality test for each cell of the design and homogeneity of variances was assessed by Levene's test. There were no outliers and residuals were normally distributed ($p > 0.05$) and there was homogeneity of variances ($p > 0.05$) with all the cases except *P. sylvestris* where the homogeneity of variances was $p = 0.022$. Non-parametric Anova was performed with *P. sylvestris* seed germination data.

The main effect and interaction effect of the independent variables pH and substrate were investigated further. If there was no interaction effect, multiple comparisons were done with Post Hoc Test with Dunnett t and Repeated Contrasts. If an interaction effect was found, the two pH levels were compared for each substrate separately. Even though there was no interaction effect detected between the independent variables, the Profile plots

revealed disordinal interaction between different levels of pH and substrate in some of the experiments. Therefore, also a simple main effect pairwise comparison was conducted if also at least one main effect was found statistically relevant. Pairwise comparisons were run through SPSS syntax command with reported +/- Standard deviation and *p*-values Bonferroni-adjusted, within each simple main effect.

There was a statistical difference between the control groups in the first lettuce seed germination experiment therefore only gauze percentages are shown in the first germination experiment results and in the second lettuce seed germination results also double filter results are shown because there was a difference between these two control groups. In the third germination experiments there was no statistical difference between the two control groups and the control gauze is shown in the results.

7 RESULTS

7.1 Germination experiment I

7.1.1 Ocium basilicum seed germination experiment

There was no interaction between pH and substrate on germination percentage of basil (p = 0.568). PH did not affect the germination of basil seeds (p = 0.284), but the average germination percentage was reduced on all *Sphagnum* substrates as compared to control gauze (p = 0.00017, Figure 3). There was a difference in mean germination percentage scores between the control gauze and all the *S.* moss substrates (Figure 5).

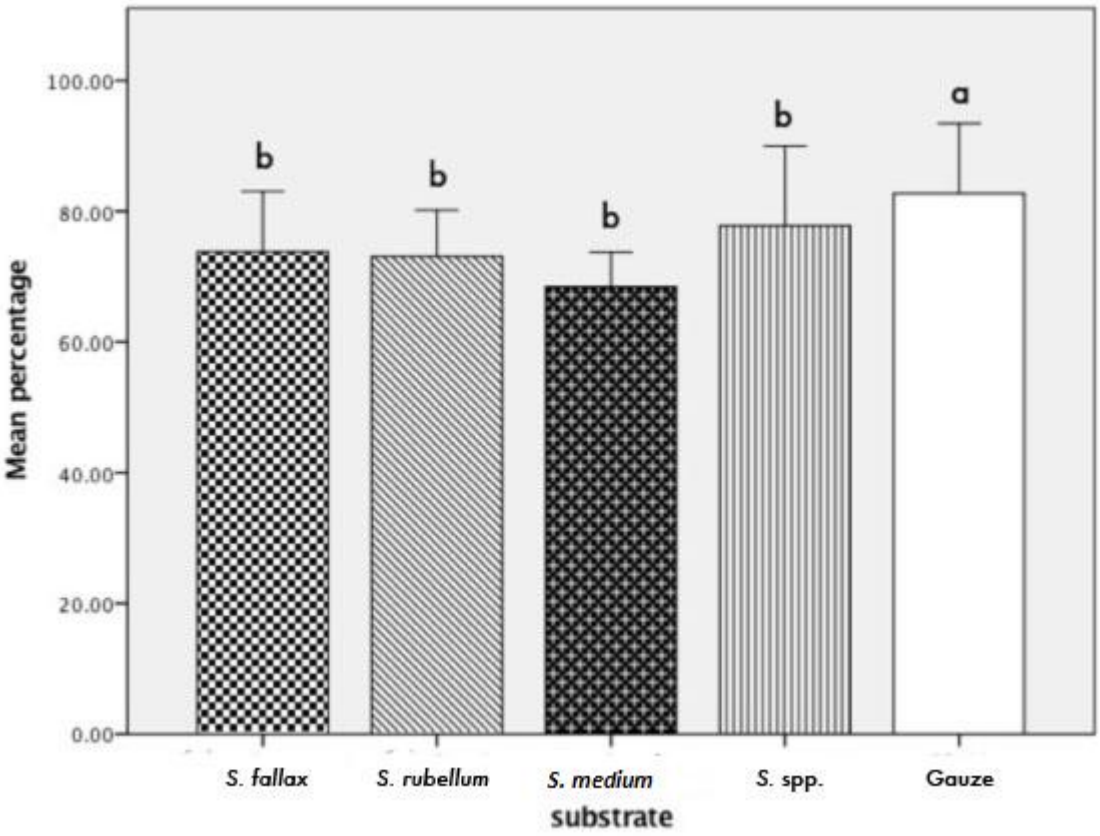


Figure 5. *Sphagnum* moss reduced basil seed germination in comparison to control gauze. The mean final germination percentages of the two pH levels are shown with error bars indicating standard deviations (n = 8). Groups marked with the same letter do not differ significantly ($p < 0.05$).

When analyzed closer, simple main effect pairwise comparison revealed that germination percentage on fresh *S. medium* was lower than on stale *S.* spp substrate (pH < 5.0) and the germination percentages on fresh *S. medium* and *S. fallax* were significantly lower than on control gauze and the other two *S.* moss substrates (pH > 5.0) (Figure 6).

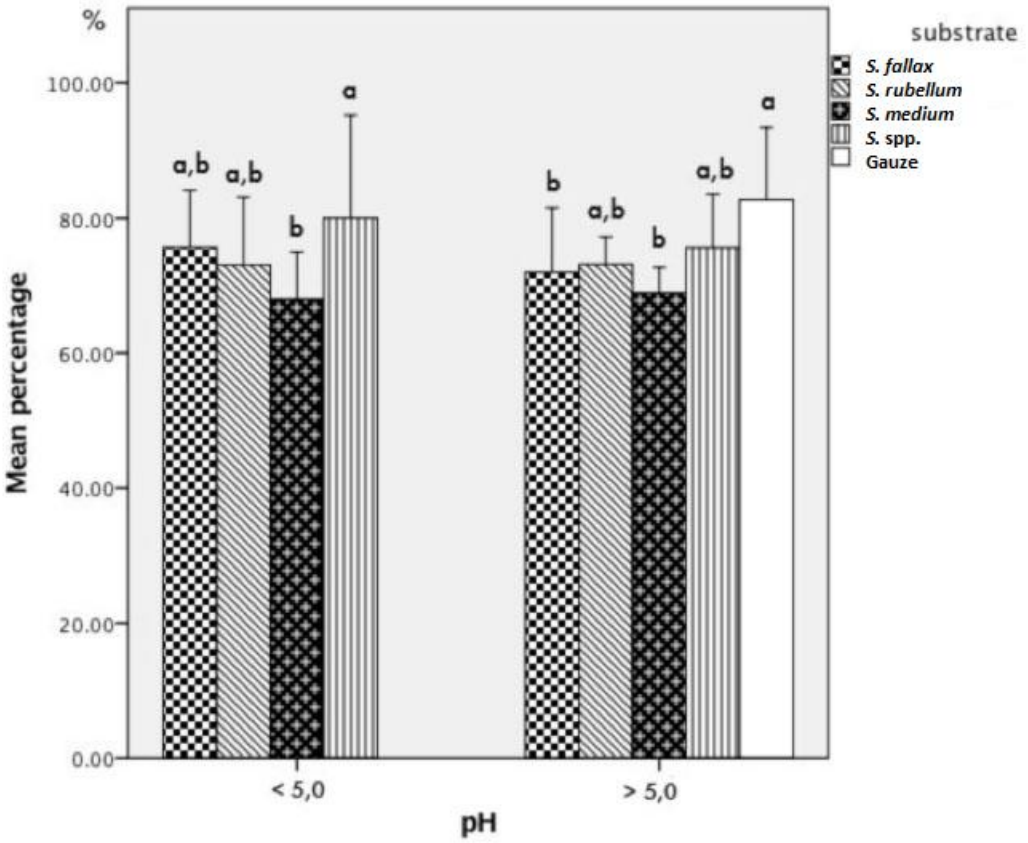


Figure 6. *S. fallax* and *S. medium* reduced basil seed germination when the substrate pH was higher than 5.0. When the substrate pH was lower than 5.0 the germination percentage was highest on *S.* spp. and lowest on S. medium substrate. The mean final germination percentages of the two pH levels are shown with error bars indicating standard deviations (n =4). Groups marked with a same letter do not differ significantly in the same pH group (p > 0.05).

7.1.2 Lolium multiflorum seed germination experiment

There was no statistically significant interaction between pH and substrate on germination percentage of ryegrass (p = 0.106). Neither pH (p= 0.256) nor substrate (p= 0.429) affected the germination of ryegrass seeds when statistically analyzed (Figure 4). The average germination percentage of the seeds on mosses treated with lime was 89,8 % and on mosses without added lime was 88,4 %. The germination percentage on the control gauze was 89.1 %.

7.1.3 Raphanus sativus var. sativus seed germination experiment

There was no interaction between pH and substrate on germination percentage of radish (p = 0.229). pH affected the germination of radish seeds (p < 0.0001) and the germination percentage was higher on pH > 5.0 substrates (65.9 %) than on pH < 5.0 substrates (58,1

%). Also, the average germination percentage was reduced on all *Sphagnum* substrates as compared to control gauze (p = 0.0015. Figure 7).

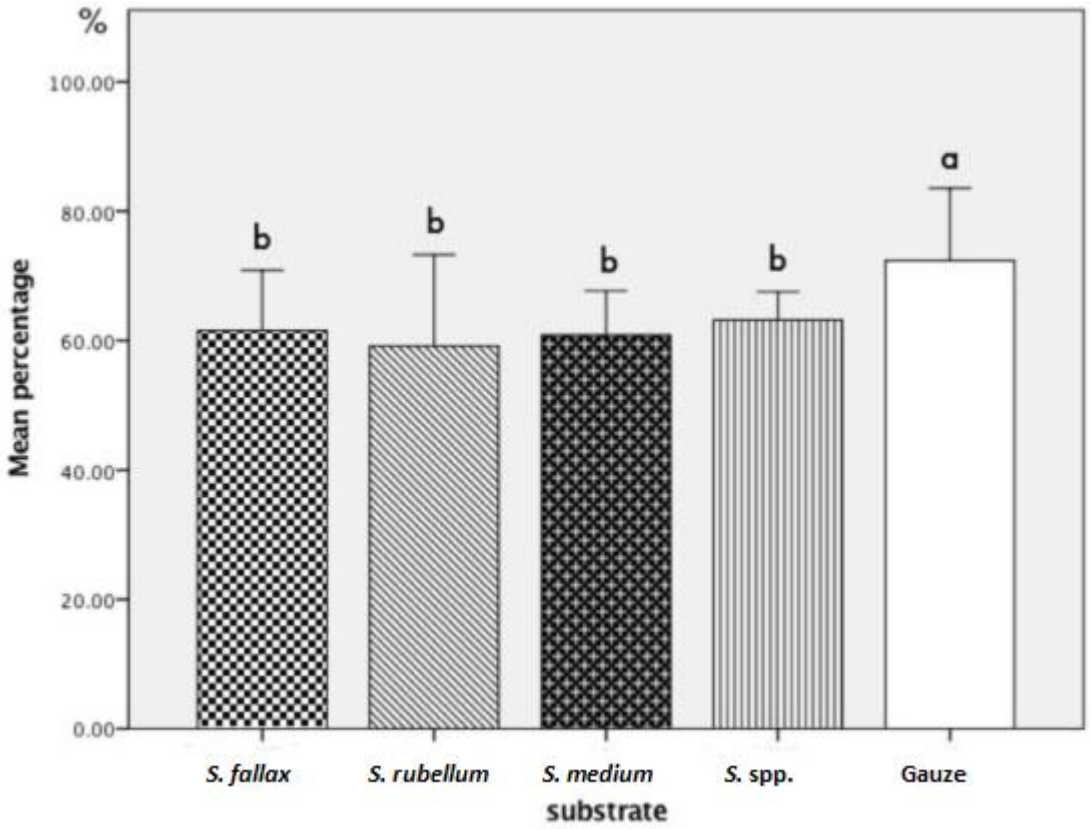
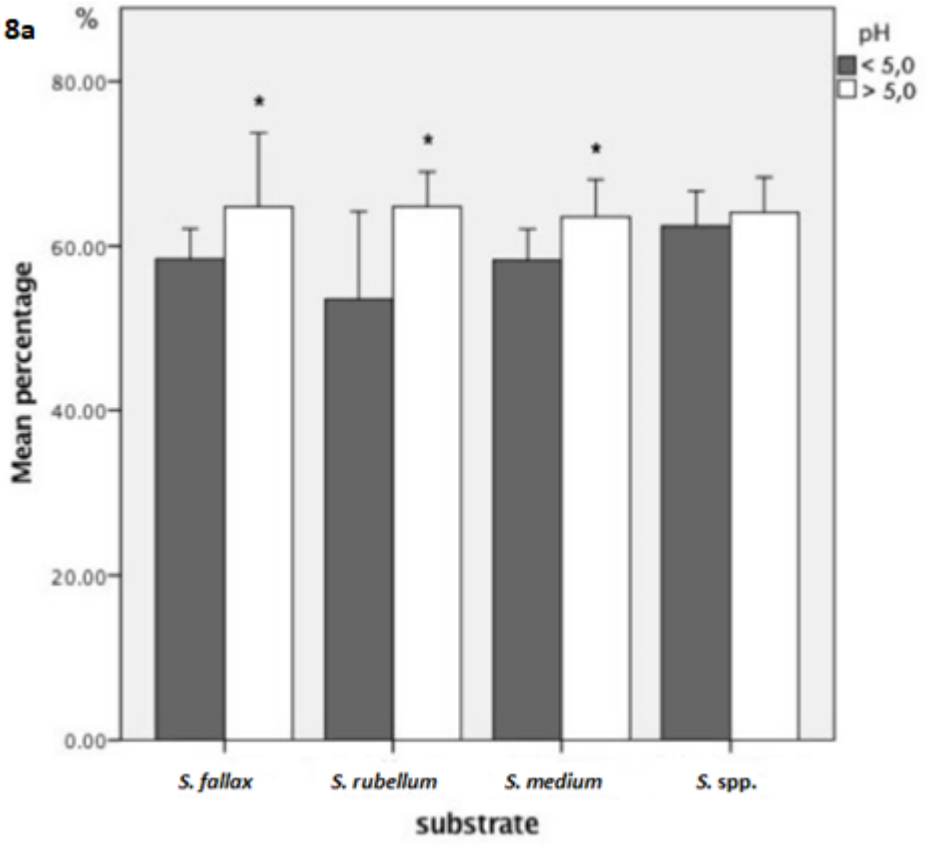


Figure 7. *Sphagnum* mosses reduced radish seed germination in comparison to control gauze. The mean final germination percentages of the two pH levels are shown with error bars indicating standard deviations (n = 8). Groups marked with the same letter do not differ significantly ($p < 0.05$).

The added $CaCO_3$ enhanced the radish seed germination in the case of the fresh mosses *S. fallax*, *S. rubellum* and *S. medium* (Figure 8a). On *S.* spp. the germination percentage was the highest and on *S. rubellum* the lowest (pH < 5.0 $p < 0.05$) (Figure 6). Germination percentage on all *S.* moss substrates was lower than on control gauze (pH > 5.0, $p < 0.05$) (Figure 8b).



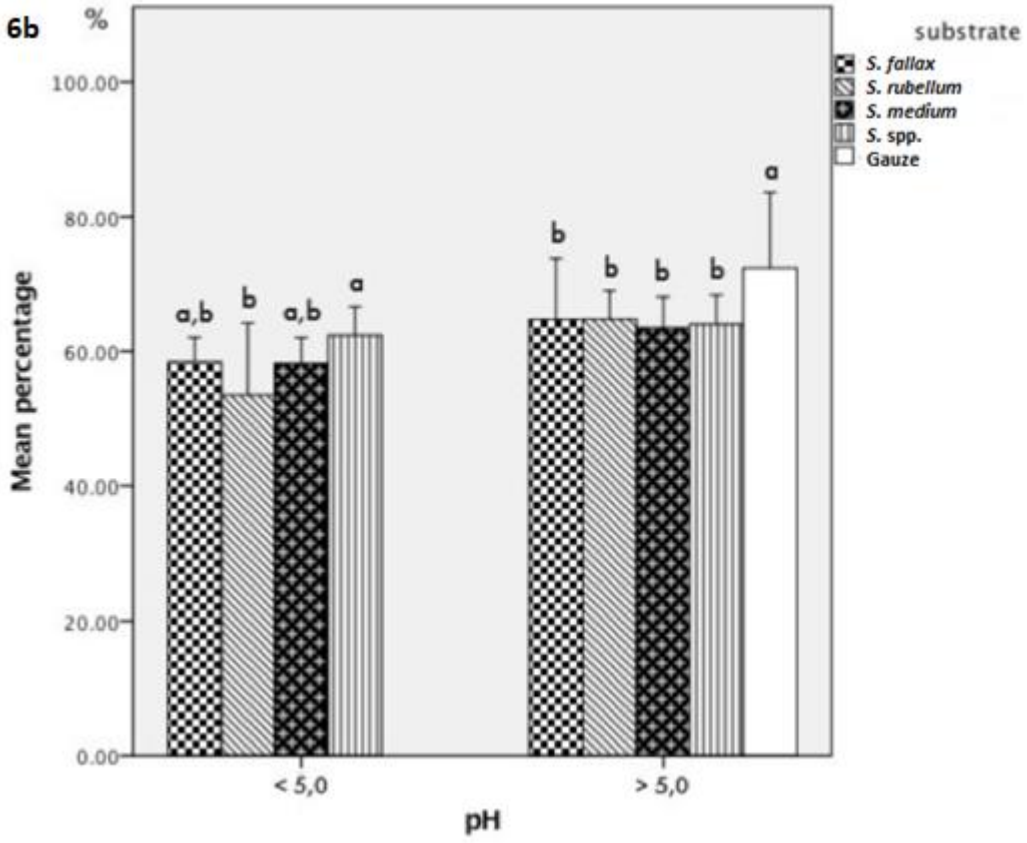


Figure 8a. Radish seed germination percentages on fresh mosses (*S. fallax*, *S. rubellum* and *S. medium*) were enhanced when the pH was over 5.0. Mean final germination percentages of the two pH levels are shown with error bars indicating standard deviations (n =4). Groups marked with * differ significantly ($p < 0.05$).

Figure 8b. All *Sphagnum* mosses reduced radish seed germination when the substrate pH was higher than 5.0. When the substrate pH was lower than 5.0 the germination percentage was highest on *S.* spp. and lowest on *S. rubellum* substrate. The mean final germination percentages of the two pH levels are shown with error bars indicating standard deviations (n =4). Groups marked with a same letter do not differ significantly in the same pH group ($p > 0.05$).

There was a difference between radish seed germination on *S. medium* with and without added $CaCO_3$ growing mediums ($p < 0.05$) and it could also be visually observed that in the case of *S. medium*, without added $CaCO_3$, germinated radish had shorter, and less vigorous roots. This negative effect caused by low pH could be seen clearly from the bottom of the petri dish at the end of the two-week experiment (Figure 9). This effect was also seen with the other *S.* moss species.

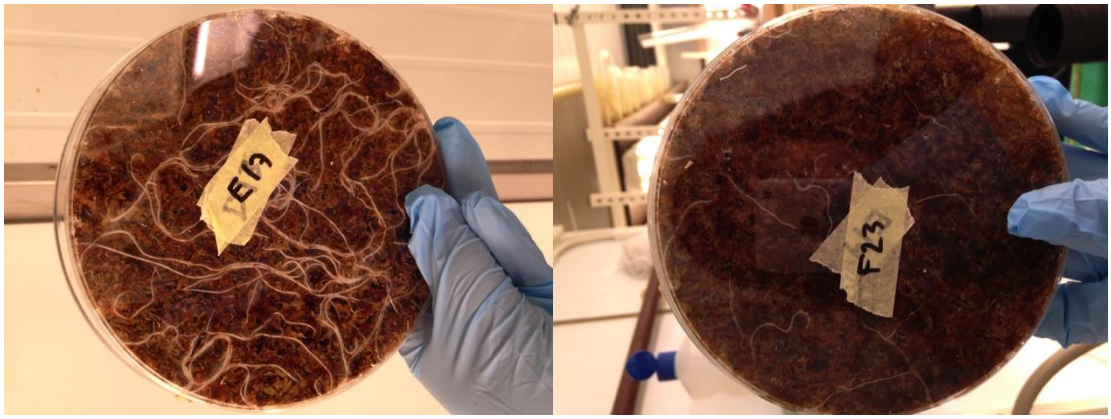


Figure 9. Radish seed germination experiment on *S. medium* with added $CaCO_3$ (left) and *S. medium* without added $CaCO_3$ (right) growing medium.

7.1.4 Pinus sylvestris seed germination experiment

For Scots pine, the average germination percentage was higher ($p = 0.005$) on pH > 5.0 substrates (86.0 %) than on pH < 5.0 substrates (81.5 %). None of the *Sphagnum* moss substrates affected the mean germination percentage as compared to control gauze ($p = 0.355$). When the moss substrates were analyzed separately, adding $CaCO_3$ enhanced pine seed germination only in the case of the “fresh” moss *S. medium* substrate ($p < 0.05$) (Figure 10).

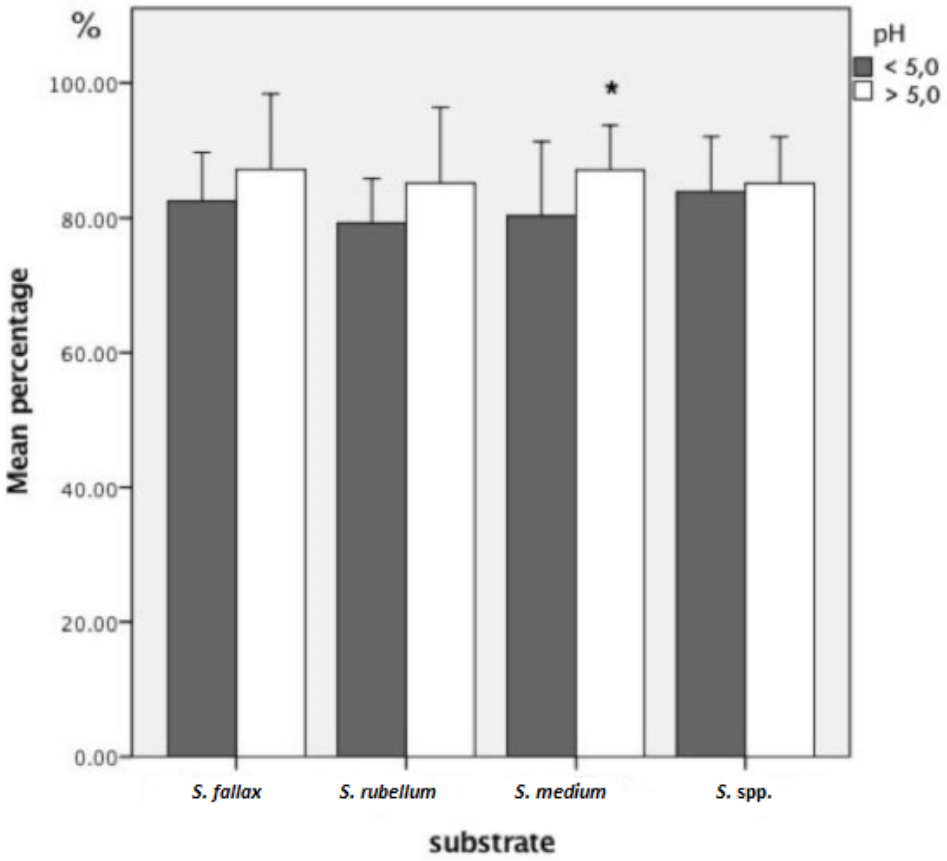


Figure 10. Pine seed germination percentage on *S. medium* was enhanced when the pH was over 5.0. Mean final germination percentages of the two pH levels are shown with error bars indicating standard deviations (n =4). Groups marked with * differ significantly ($p < 0.05$).

7.1.5 Lactuca sativa seed germination experiment

There was no interaction between pH and substrate on germination percentage of lettuce ($p = 0.557$). pH did not affect the germination of lettuce seeds ($p = 0.225$), but the average germination percentage was reduced on all *Sphagnum* substrates as compared to controls double filter paper and gauze ($p < 0.0001$) (Figure 11). Also, there was difference between the two controls ($p = 0.001$) (Figure 9). From the *S.* mosses, on *S. fallax* substrate the germination percentage was the highest ($p < 0.05$) (Figure 11).

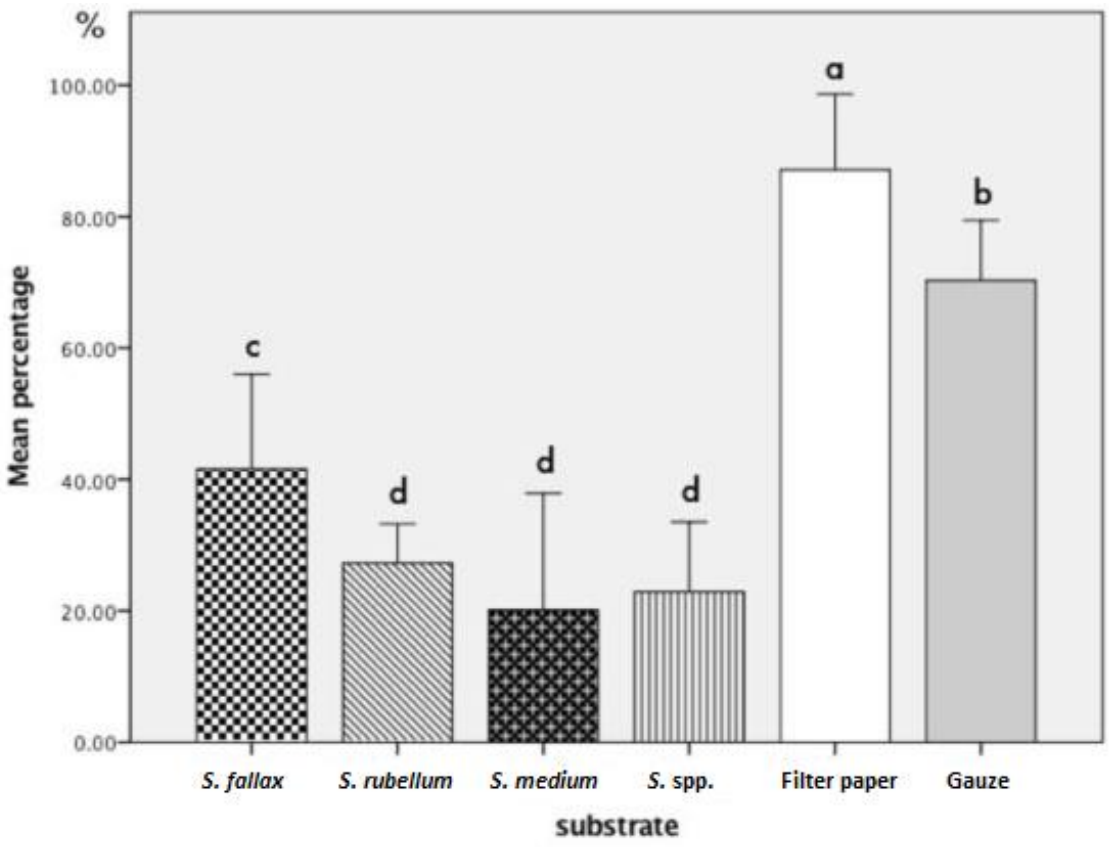


Figure 11. *Sphagnum* mosses reduced lettuce seed germination in comparison to control filter paper and gauze. Also, the germination percentages on two different control treatments differed. The mean final germination percentages of the two pH levels are shown with error bars indicating standard deviations (n = 8). Groups marked with the same letter do not differ significantly ($p < 0.05$).

There was a difference between germination percentage on *S. fallax* and on other *S.* moss substrates (pH < 5.0, $p < 0.05$) (Figure 12). Also, the germination percentage on *S.* moss substrates was lower than on control double filter paper (pH > 5.0, $p < 0.05$) (Figure 12).

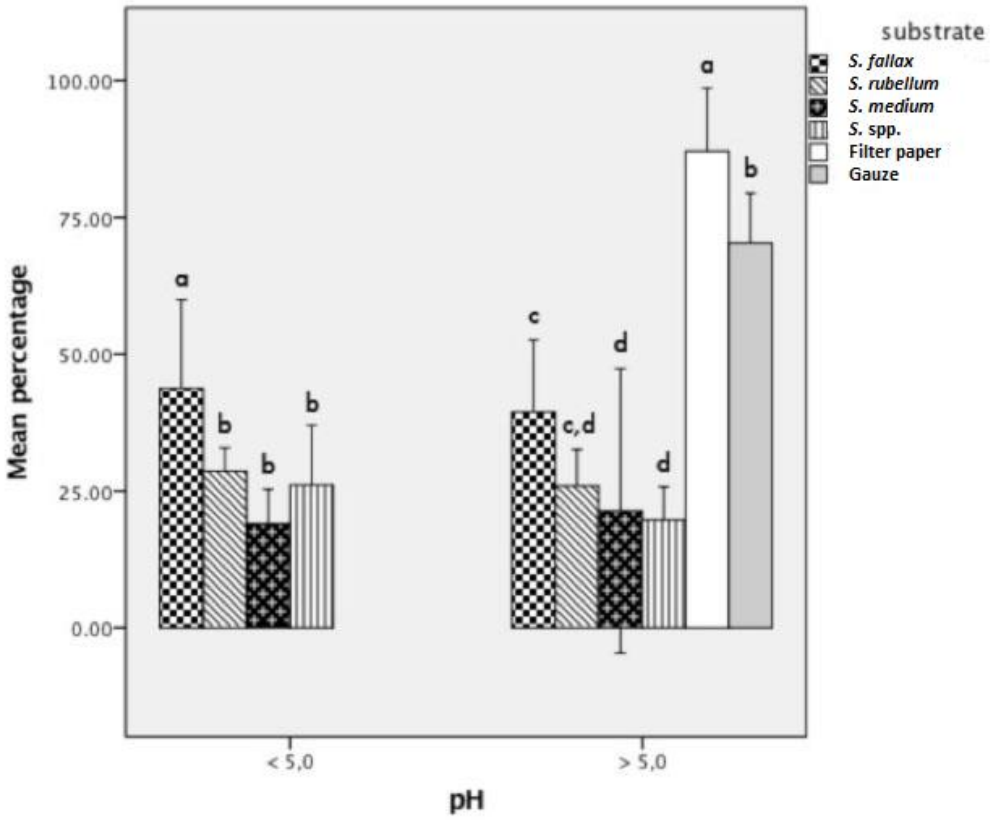


Figure 12. All *Sphagnum* mosses reduced lettuce seed germination when the substrate pH was higher than 5.0 when compared to the both controls filter paper and gauze. When the substrate pH was lower than 5.0 the germination percentage was highest on *S. fallax*. The mean final germination percentages of the two pH levels are shown with error bars indicating standard deviations (n =4). Groups marked with a same letter do not differ significantly in the same pH group ($p > 0.05$).

Even though there was no difference between lettuce seed germination on *S. medium* or *S. medium* with added $CaCO_3$ growing medium ($p = 0.598$) it could be visually observed that *S. medium* without added $CaCO_3$ had shorter, thicker roots with a hint of brown in the radicula. This effect was also seen with the other S. mosses with and without added lime. This negative effect caused by low pH could be seen clearly when counting the germinated seeds (Figure 13).

Figure 13. Lettuce seeds grown on *S. medium* with added $CaCO_3$ (left) and *S. medium* without added $CaCO_3$ (right) growing medium.

7.2 Germination experiment II - Second *Lactuca sativa* seed germination experiment

There was no interaction between pH and substrate on germination percentage of lettuce ($p = 0.965$). The pH-level affected the germination of lettuce seeds ($p = 0.025$), and the average germination percentage was reduced on all *Sphagnum* substrates as compared to controls double filter paper and gauze ($p < 0.0001$, Figure 14).

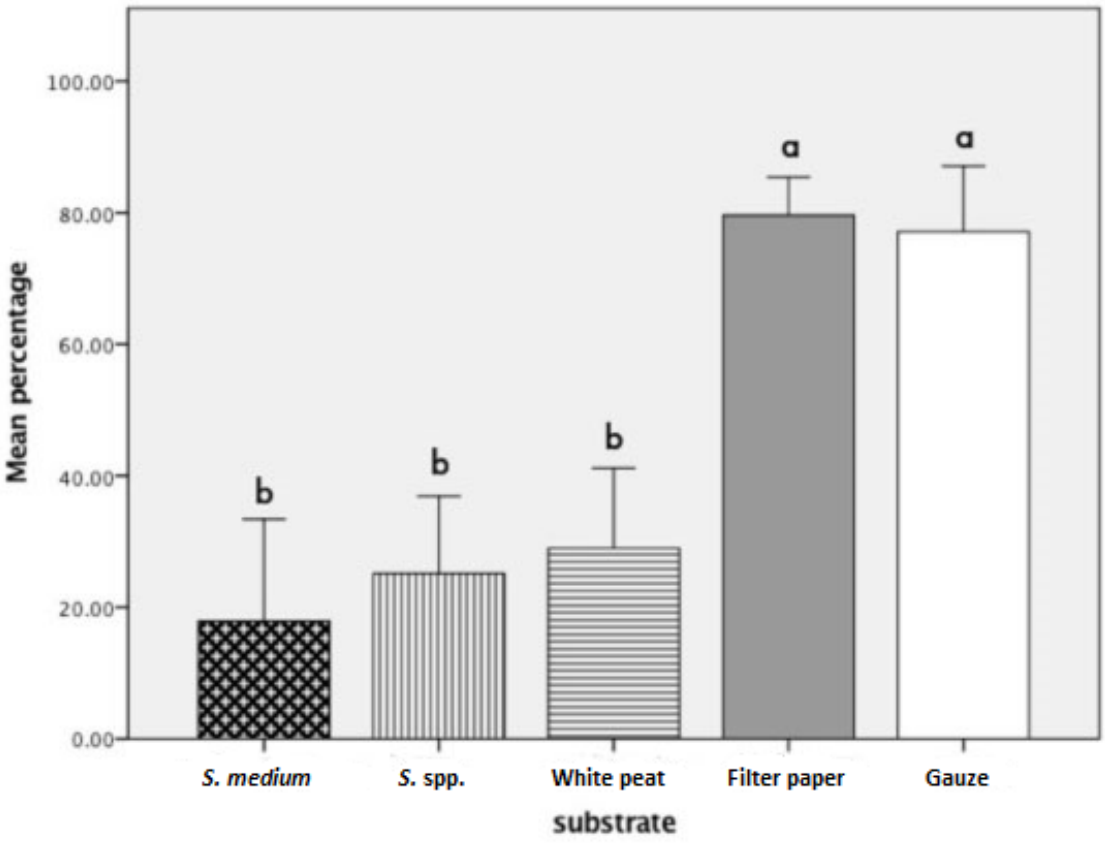
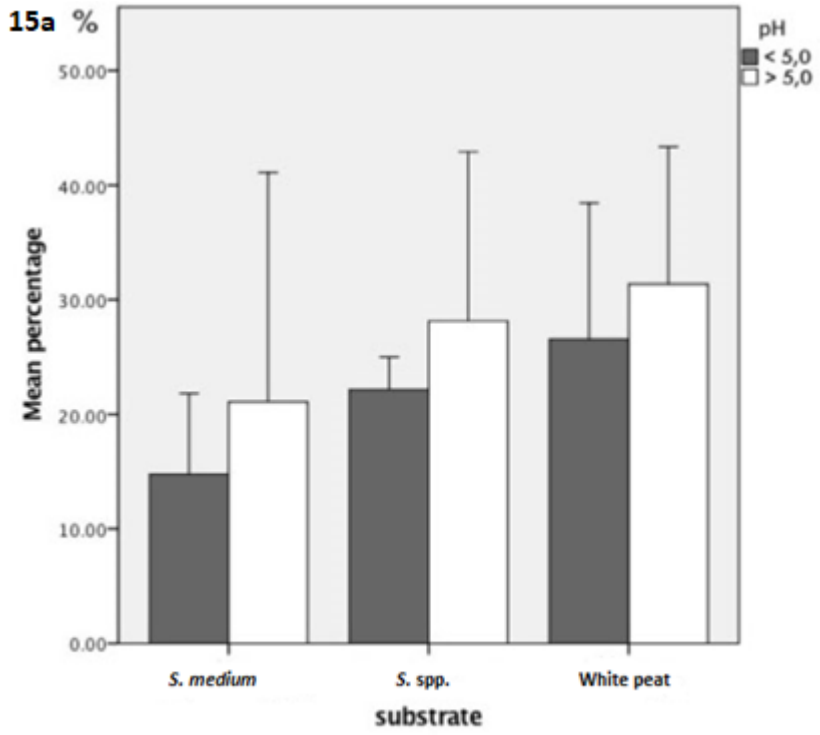


Figure 14. *S. medium*, *S.* spp and white peat reduced lettuce seed germination in comparison to control filter paper and gauze. The mean final germination percentages of the two pH levels are shown with error bars indicating standard deviations (n = 8). Groups marked with the same letter do not differ significantly ($p < 0.05$).

There was no a difference between germination percentage on white peat and on *S.* moss when lime was added on substrates (Figure 15a). There was a difference between germination percentage on control double filter paper and on *S.* moss substrates (pH > 5.0, $p < 0.05$) (Figure 15b). On organic substrates without added lime the germination percentage was highest on white peat and lowest on *S. medium* ($p < 0.05$) (Figure 13b). Germination on control double filter paper started on the second day and on *S.* moss and white peat substrates on the fourth day of the experiment.



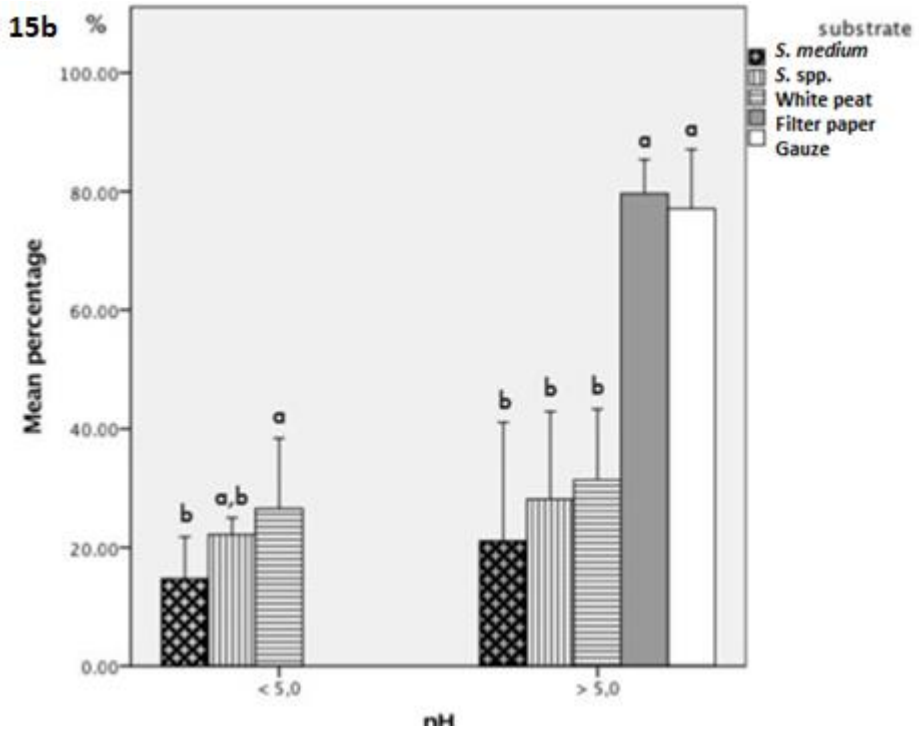


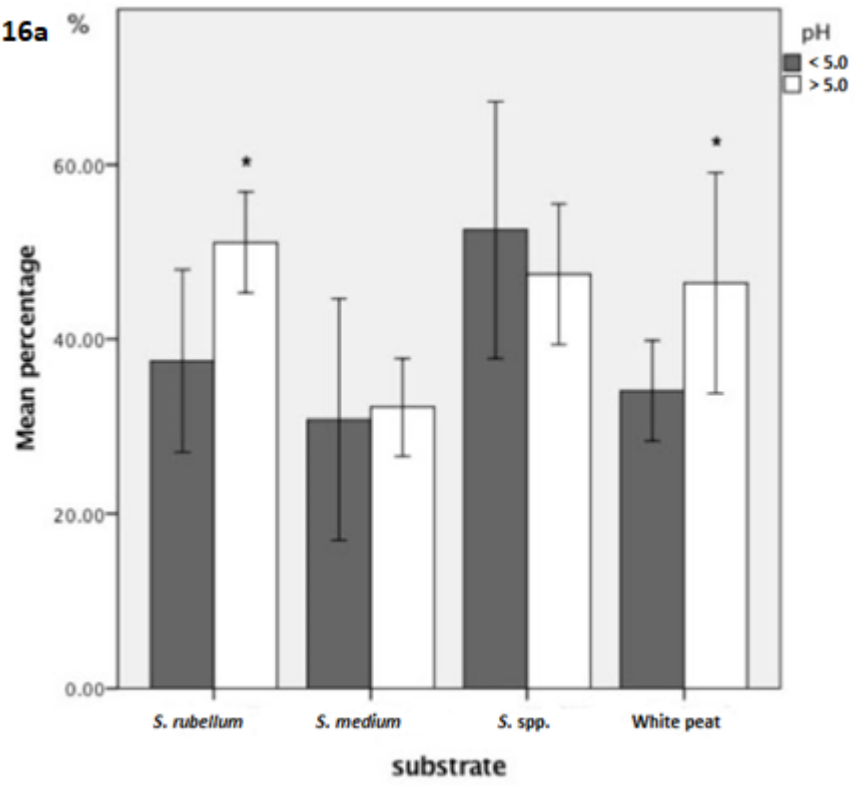
Figure 15a. Lettuce seed germination was not enhanced by added lime on *S. medium*, *S.* spp. and white peat (pH > 5.0). Mean final germination percentages of the two pH levels are shown with error bars indicating standard deviations (n =4). Groups marked with * differ significantly ($p < 0.05$).

Figure 15b. All *Sphagnum* mosses reduced lettuce seed germination when the substrate pH was higher than 5.0. When the substrate pH was lower than 5.0 the germination percentage was highest on white peat and lowest on *S. medium* substrate. The mean final germination percentages of the two pH levels are shown with error bars indicating standard deviations (n =4). Groups marked with a same letter do not differ significantly in the same pH group ($p > 0.05$).

7.3 Germination experiment III - Third *Lactuca sativa* seed germination experiment

There was an interaction between pH and substrate on germination percentage of lettuce ($p = 0.002$). The main effect substrate was statistically significant ($p < 0.001$).

The added $CaCO_3$ enhanced the lettuce seed germination in the case of the *S. rubellum* and white peat (Figure 16a). The germination percentage on *S. spp.* was higher than on other *S.* moss substrates (pH < 5.0, p < 0.05) (Figure 16b). Also, there was a difference between germination percentage on control gauze and on organic substrates (pH > 5.0). On *S. medium* substrate the germination percentage was the lowest (pH > 5.0, p < 0.05) (Figure 16b).



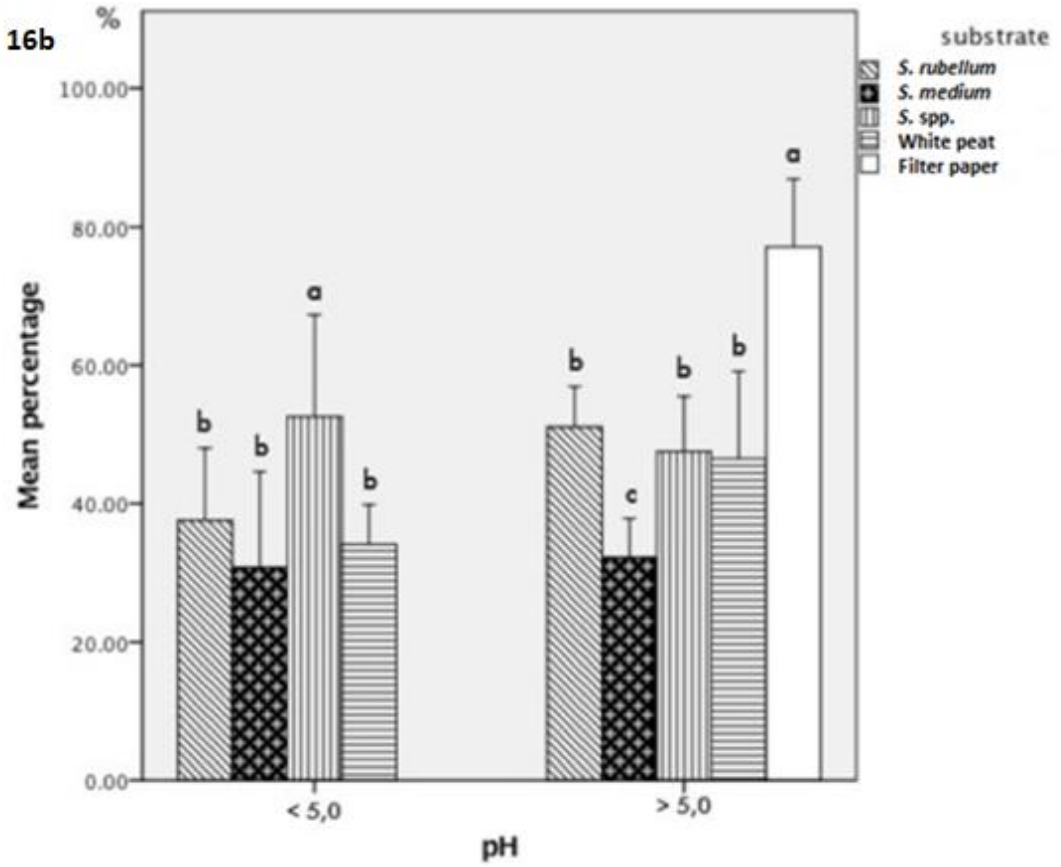


Figure 16a. Lettuce seed germination was enhanced by added lime on *S. rubellum* and white peat (pH > 5.0). Mean final germination percentages of the two pH levels are shown with error bars indicating standard deviations (n =4). Groups marked with * differ significantly ($p < 0.05$).
Figure 16b. All *Sphagnum* mosses and white peat reduced lettuce seed germination when the substrate pH was higher than 5.0. When the substrate pH was lower than 5.0 the germination percentage was highest on stale moss *S.* spp. The mean final germination percentages of the two pH levels are shown with error bars indicating standard deviations (n =4). Groups marked with a same letter do not differ significantly in the same pH group ($p > 0.05$).

The third lettuce seed germination experiment done with moss water extracts seemed to enhance the germination on all the organic growing mediums when compared to the two previous experiments (Table 5).

Table 5. Mean germination percentages of *Sphagnum* mosses that were present in all the three *L. sativa* germination experiments, white peat and double filter paper.

pH > 5.0	1st germination %	2nd germination %	3rd germination %
S. medium	21,3	21,1	32,2
S. spp.	19,7	28,1	47,5
White peat	-	31,4	46,5
Filter paper	87,1	79,6	77,0

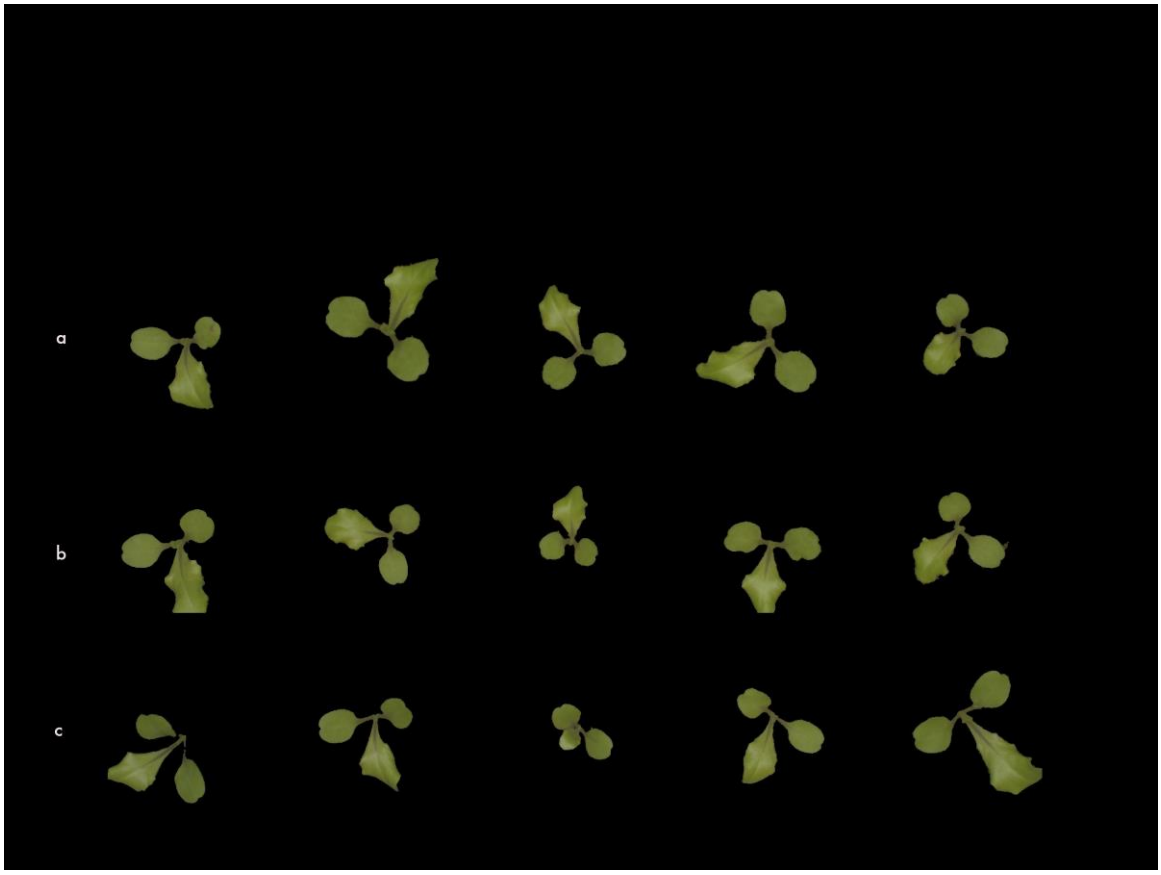
7.4 *Lactuca sativa* seedling growth experiment

The only difference between lettuce seedling development on different substrates was found in the beginning of the experiment measurements in NaPPi 28.03.2017, when the surface area of *L. sativa* 'Australische gele' grown on white peat substrate was measured to be statistically significantly lower than when grown in *S. medium* or commercial peat substrate. When the measurements were taken six days later 03.04.2017 the same difference was found again between white peat and commercial peat, but not with *S. medium* substrate. This was the last day of reliable measurements, because after this the lettuce leaves started to grow over the pot edges and on top of each other. At the end of the experiment there was no difference ($p > 0.05$) in the means of fresh weight, dry weight and leaves of *L. sativa* 'Australische gele' or *L. sativa* 'Grand rapids' grown on commercial peat, *S. medium* and white peat substrate (Table 6).

Table 6. Influence of the substrate on two lettuce cultivar dry and fresh weights. Cpm: commercial peat substrate, Sm: *Sphagnum medium* and Wp: white peat. No differences were found between the substrates ($p > 0.05$).

Cultivar	Substrate	Canopy dry weight, g plant (n=10)	Canopy fresh weight, g plant (n=10)
Australische gele	Cpm	0,11	2,51
Australische gele	Sm	0,11	2,42
Australische gele	Wp	0,10	2,11
Grand rapids	Cpm	0,10	2,26
Grand rapids	Sm	0,10	2,09
Grand rapids	Wp	0,10	2,11

The both lettuce cultivars were normally developed in all the substrates and no abnormality in the shape or phytotoxity symptoms was observed in the case of *L. sativa* 'Grand rapids' and 'Australische gele' grown on *S. medium* substrate (Figure 17).



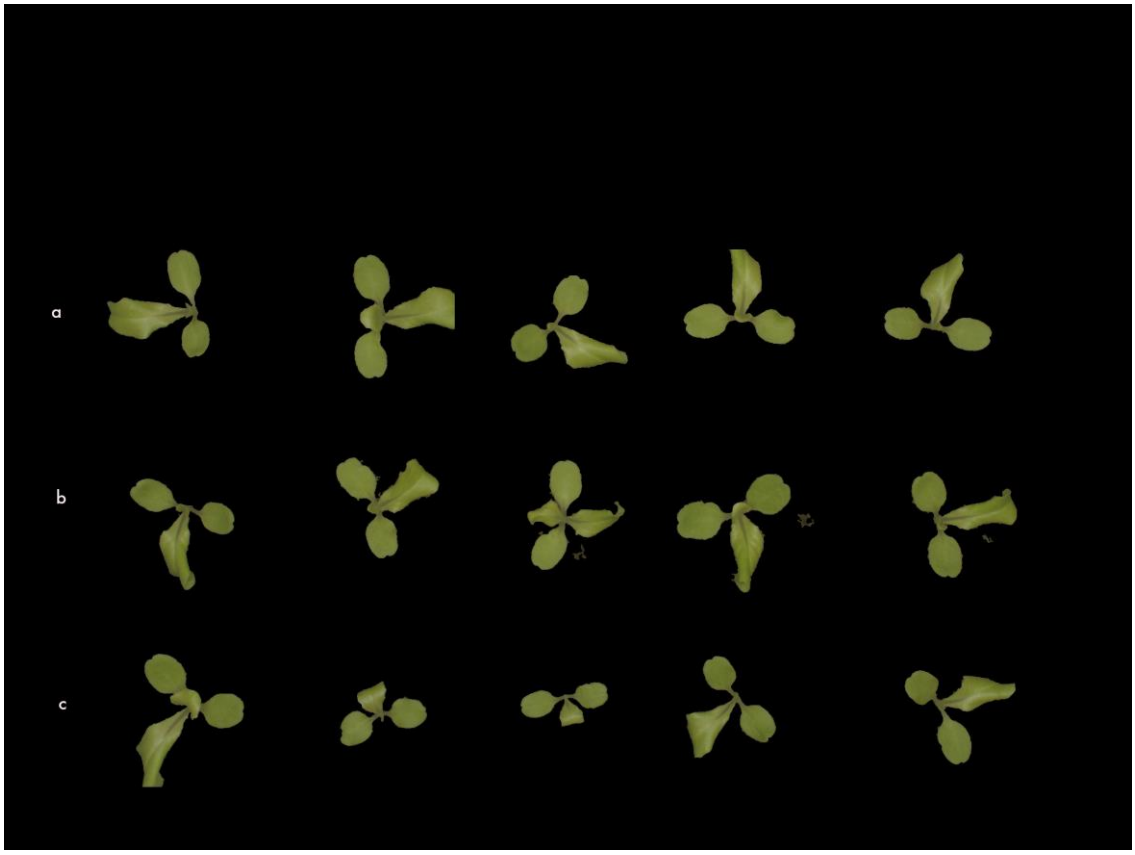


Figure 17. RBG image of *L. sativa* seedling development, picture taken on day 9 (02.04. 2017) after sowing the seeds (24.03. 2017). Above *L. sativa* 'Grand rapids' and below *L. sativa* 'Australische gele'. a: commercial peat substrate
b: *S. medium* substrate c: white peat substrate

8 DISCUSSION

8.1 Allelopathic effect

S. mosses showed seed germination inhibition in the cases of dicotyledon species, but not in the case of monocotyledon and gymnospermous species and it was confirmed that indeed *S.* mosses can cause unexplained germination inhibition, but this inhibition is very donor and receiving plant specific and not only caused by the low pH of the *Sphagnum* substrate.

8.2 Effects of low pH on vascular plant seed germination

Only in the cases of radish and pine the added $CaCO_3$ enhanced the germination percentage on some of the *Sphagnum* moss substrates, and after adding $CaCO_3$ only pine seed germination percentage was on the same level as the control group germination percentage. Therefore, it can be reasoned that putative allelopathic effect is not only caused by the low pH, at least not in the case of dicotyledon seed germination, and other factors should be taken into consideration. One factor that might have negatively affected the seed germination or at least delayed it is the physiological properties of *S.* moss fibers. *S.* mosses good water holding capacity could have prevented the seeds to get enough water for the germination in the two first experiments. Also, the micro-orcanisms (bacteria and fungi) present in the *Sphagnum* moss fibers could have affected the seed germination and not the moss itself, as Frahm et al. (2012) discussed in their study of bryophyte allelopathy on garden cress *Lepidium sativum* and lettuce *Lactuca sativa*. Then again, at the last lettuce seed germination experiment only water extracts were used, and the germination inhibition was still shown. Therefore, this inhibition is most likely related to *Sphagnum* mosses special chemical composition that causes allelopathic effect.

8.3 Allelopathic effect related to seed morphology

From the five vascular plant species studied in the first germination experiments basil, radish, and lettuce, the dicotyledon species showed germination inhibition when grown on *S.* moss substrates. The monocotyledon species ryegrass and the gymnospermous species pine didn’t show germination inhibition when grown on *S.* moss substrates.

Therefor it can be concluded that the dicotyledon seeds are more susceptible for putative allelopathic effect caused by *S.* mosses. In the beginning of the study it was suspected that some of the *Sphagnum* mosses phenol compound are responsible of the possible putative allelopathic effect. After the experiments showed clearly that only diocotyledon species were affected by *Sphagnum* moss substrates and the literature review revealed that the amounts of phenol compounds in *Sphagnum* mosses were estimated to be very small, too small to inhibit germination, also other alternatives had to be considered.

In a recent study perlatolic acid and derivatives were tested for their allelopathic effect on weeds, with lettuce (*L. sativa*) and onion (*Allium cepa L.*, Peres et. al. 2016). In the study it was concluded that the ester iso-propyl and sec-butyl 2-hydroxy-4-methoxy-6-n-pentylbenzoate (6 and 8) could be used as model molecules in the research of herbisides for dicotyledon species and n-pentyl 2-hydroxy-4-methoxy-6-n-pentylbenzoate (10) might be used for monocotyledon species (Peres et al. 2016). However, there are no studies of *Sphagnum* mosses and perlatolic acid. At Peres et al. (2016) study the perlatoric acid was acquired from lichen *Cladina confuse.*

In another study lupine triterpenes from bioactive fractions of sweet clover (*Melilotus messanensis*) were tested for allelopathic seed germination inhibition in the case of dicotyledon species lettuce (*L. sativa*) and garden cress (*Lepidium sativum*) and in the case of monocotyledon species barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*, Macias et al. 1994). In this study lupine triterpenes were found to possess potential allelopathic activity particularly in the case of dicotyledon species (Macias et al. 1994). Also, in the case of sunflower (*Helianthus annuus L.*) guaianolides (sesquiterpene lactones) extracted from the leaves inhibited particularly dicotyledon lettuce (*L. sativa*) seed germination and not so much monocotyledon barley (*Hordeum vulgare*) seed germination (Macias et al. 1993). Already in 1958 Ives and O'Neil extracted tritriterpenes (α-amyrin, taraxerone and taraxerol) from *Sphagnum* peat moss. Further study of *Sphagnum* mosses triterpenes might shed more light to the allelopathic effect shown in this study.

8.3.1 *O. basilicum* and allelopathy

In the case of basil, no supportive or unsupportive research literature was found where basils sensitivity to allelochemicals were tested. Then again only two of the tested *Sphagnum* species showed inhibition in the seed germination experiment, and the other

two did not. It is uncertain if this shown allelopathic effect is caused by allelochemicals or *Sphagnum* mosses strong water holding capacity.

8.3.2 *R. sativus var sativus* and allelopathy

In the case of radish seed germination *Sphagnum* moss substrate with and without added lime all the substrates inhibited seed germination significantly. This is totally contradictory to the results Chiapusio et al. (2013) found out in their study where *S. fallax* and *S. medium* had no effect on radish seed germination. Then again, the radish variety was not revealed in the study and they also excluded the low pH as a contributing factor by having a control with the same lower pH (Chiapusio et al. 2013). In this study raising the pH had a significant effect on seed germination in the case of the fresh mosses *S. fallax*, *S. rubellum* and *S. medium*.

Other studies have been more successful in determining allelochemicals, also found in *Sphagnum* mosses, negative effects on radish seed germination. In a study by Einhellig (1978) 2,5x 10^{-3} vanillic acid treated radish seeds had the germination percentage of 71 %. 2,5x 10^{-3} *p*-hydrobenzoic acid treated radish seeds had the germination percentage of 95 % (Einhellig 1978). When these two compounds with the same concentration were combined the germination percentage of radish seeds was 52 % (Einhellig 1978). According to Rasmussens (1995) study *S. magellanicum* (1.6×10^{-8} M) and *S. fallax* (4.5×10^{-8} M) don't consist of enough watersoluble *p*-hydrobenzoic acid to be responsible of inhibition shown in this study. Then again, it cannot be totally ruled out that the combination of *Sphagnum* moss phenolic compounds could be responsible for the allelopathic effect shown in the experiment. Also, coumarin and 2-hydroxycinnamic acid have been found to cause seed germination inhibition in the case of radish (Aliotta et al. 1993) but yet again, it is uncertain that the *Sphagnum* mosses possess necessary amounts of these compounds.

In the case of phenols *sphagnum* acid that was the largest group of phenolic compounds produced by *S. magellanicum* 1.334×10^{-6} M and *S. fallax* 1.769×10^{-6}, could be the cause of inhibition shown in the experiment (Rasmussen 1995) by itself or together with some other phenolic acid. Yet again, the amount of sphagnum acid seems quite low to be responsible of the germination inhibition shown in the experiments.

8.3.3 L. sativa and allelopathy

The inhibition shown in all the seed germination experiment done with *L. sativa* on *S.* moss and white peat growing mediums with added lime leads to the conclusion that the lettuce seed in particularly sensitive to the allelopathic effect caused by *S.* moss growing mediums. Then again lettuce seed is commonly used in allelopathy research because of its sensitivity to secondary metabolite products. The inhibition was strongest in the case on *S. medium*, but at least part of this inhibition could be explained with mosses physical properties like strong water holding capacity. In the first two seed germination experiment done with *S.* moss fiber and white peat the seed germination started one-two days later than control groups and when done with only moss water extract this difference disappeared. This indicates that pure moss water extract increases the germination percentage, probably caused by better water availability.

Lower germination percentages of *L. sativa* on *S.* moss substrates, could also be explained by the phenol compounds found in *S.* mosses by Rasmussen (1995). In a study by Li et al. (1993) it was found that *trans*-Cinnamic acid and *p*-Coumaric acid inhibited lettuce seed germination little at amount of 10^{-3} M or lower but had strong inhibition activity when the concentrations were higher. In the study the *L. sativa* germination test on filter paper treated with *trans*-Cinnamic acid the germination percentage of *L. sativa* was 28 +/- 15 %, and on *p*-Coumaric acid treated filter paper 52 +/- 10 % (Li et al. 1993). When these two compounds were combined germination inhibition was even greater (Li et al. 1993). Then again, the amounts of *trans*-Cinnamic acid and *p*-Coumaric acid found in *S. fallax* and *S. magellanicum* by Rasmussen (1995) were much lower (1.8×10^{-8}- 2.0×10^{-8} M) than those used in the study by Li et al. (1993).

In another study *p*-hydrobenzoic acid was studied for the cause of *L. sativa* seed germination inhibition (Chiapusio 1997). In the two-day experiment the strongest concentration 10^{-3} M of hydrobenzoic acid didn't show inhibitive effect on *L. sativa* seed germination (Chiapusio 1997). According to Rasmussen (1995) findings, the amounts of hydrobenzoic acid are much lower **(**1.6×10^{8}- 4.5×10^{8} M) in *S. fallax* and *S. magellanicum* and therefore it is very unlikely that the germination inhibition shown in this experiment would be caused by *p*-hydrobenzoic acid.

There are no studies made of *Sphagnum* moss, species specific, phenol compound *sphagnum* acid and its modes of action in relation to allelopathic effect. This phenol compound forms the largest part of organochemical compounds excreted from *S. fallax* and *S. magellanicum* (Rasmussen et al. 1995) and therefore the amounts are on the right scale (10^{-3}-10^{-9} M) of tested putative allelochemical concentrations, that have previously been found to have inhibitory effect on seed germination. Then again, the phenol amounts in *S.* moss species used in these experiments were not measured and the amounts of phenols are known to vary greatly according to the season and the environment.

In the second and third lettuce seed germination experiment white peat product without added lime or fertilizers caused seed germination inhibition. In a study by Bragazza and Freeman (2007) newly formed *Sphagnum* litter peat collected from 2-4 cm below the capitulum revealed the polyphenol amounts in the newly formed peat layer to be with lawn species (*S. magellanicum*) 0.32 mg g^{-1}. The study also showed that increase in atmospheric N leads to increase in N amounts in litter peat and corresponds to decrease in soluble polyphenols (Bragazza and Freeman 2007). Then again according to Jassey et al. (2011) at least the polyphenol amounts of *S. fallax* moss are highest in the moss capitulum area and lower in the lower parts of the moss. It can't be ruled out that there are no active polyphenols in the newly formed peat that could be responsible for the shown seed germination inhibition.

8.3.4 L. multiflorum and allelopathy

There are some studies showing that ryegrass is susceptible to allelopathic effect caused by vascular plant species like barley flower *Parthenium hysterophorus* (Mersie and Singh 1986) and bryophyte species like *S. fallax* and *S. magellanicum* (Chiapusio et al. 2013). In their study though the tested ryegrass species was *Lolium perenne* a different ryegrass species that was used in this study (Chiapusio et al. 2013). Then again, the study by Chiapusio et al. (2013) found Raphanus sativa seed germination was not inhibited by the *Sphagnum* species *S. fallax* and *S. magellanicum* but the root growth was supressed. The reason for these contradictory results can be in the time of the year when *Sphagnum* samples were collected. The amount of secondary metabolite products produced by *S.* mosses are known to vary between the seasons.

8.3.5 P. sylvestris and allelopathy

In the case of pine, no seed germination inhibition was seen in the experiments where *Sphagnum* mosses were used as a substrate. This was well expected because pine (*Pinus sylvestris*) is a common plant in the bog environments where these specific *Sphagnum* mosses grow. Usually the pines grow in the outskirts of the raised bogs and/or on top of the hummocks that are formed in the bog environment.

8.4 Variability in different *Sphagnum* moss species allelopathic effect

Seeds germinated on different *S.* moss species showed great variability in the case of diocotyledon species basil, radish and lettuce. From the lime treated substrates basil germination percentage was lowest on *S. fallax* and *S. medium*, radish germination percentage was lowest on all the *S.* mosses and lettuce seed germination percentage was lowest on *S. medium* and *S. spp.* According to this, it can be argued that *S. medium* might not be best option for growing substrate. Then again, this inhibitive effect can also be partly caused by *S.* mosses special physical properties like bigger size and better water holding capacity.

Also, the long storage time didn't remove the allelopathic effect entirely in the case of *S.* spp versus the fresh mosses. On the long-stored lime treated stale *Sphagnum* spp. substrate the germination percentages of basil were similar with the control group and this might suggest that the allelopathic compounds could have lost their effect during the long storage time. Then again germination percentage of basil germinated on *S. fallax* substrate didn't differ from the control either. Therefore, it is too early to draw any conclusions of this, particularly when the germination percentage of lettuce (1st experiment) on *S. spp.* was the lowest together with S. *medium* when compared to the other S. mosses and controls.

8.5 Neutral allelopathic effect of *Sphagnum* moss on *L. sativa* seedling development

The main cause for not seeing any negative allelopathic effect in the *L. sativa* seedling growth experiment was most likely caused by a weaker concentration of allelochemicals in the surface of the substrate. This was due to watering the substrate from the surface, from where the potential allelochemicals were dissolved to the lower parts of the substrate. The *Sphagnum* moss fibers were so loosely backed in the growing pots that the capillary water potential wasn't functioning. This led to uneven concentration levels in the substrate that were not evened out.

The other possible reason for not seeing the allelopathic effect is related to chemical reactions in the substrate. The action of allelochemicals (including phenolics) can be interrelated with nutrient conditions, with high concentrations of N, K, P and phosphate altering allelopathic activities (Inderjit and del Moral 1997, Reigosa and Pazos-Maldivo 2007). In their study about allelochemicals effect on *Arabidopsis thaliana*, Reigosa and Pazos-Maldivo concluded that when the nutrients were present in the liquid solution, a higher concentration was necessary to obtain a reduction of 50 % in root growth than without nutrients. This might be the explanation for the allelopathic effect not shown in the *L. sativa* seedling growth experiment, where the seeds germinated and grew normally on the *S. medium* substrate. Even though nutrients are not needed for germination, it could be speculated that they reacted with the *Sphagnum* moss secondary metabolite products that inhibited the seed germination in the experiments done with *L. sativa*.

9 CONCLUSIONS

9.1 Practical aim of the study

The practical aim of the study was to find out how feasible it is to use *Sphagnum* moss as a part of horticultural growth substrate and if there were observations made of germination and seedling growth inhibition. In the case of *L. sativa* strong inhibition of germination was seen when grown on *Sphagnum* moss growing mediums. This leads to the conclusion that some unknown allelochemicals, one or mixture was at work here. Also, prolongation of the germination was observed when germinated on *Sphagnum* fibers. This was thought to be caused by the strong water holding capacity of *S.* moss. Then again, in the real-life growing conditions, all the evidence of the germination inhibition and seedling growth inhibition disappeared. This is thought to be caused by watering the substrate which dilutes the allelochemicals so that the concentration is no longer harmful for the seed germination and the seedling development. In the basis of this study there is no obstacle seen in using *Sphagnum* moss as a part of horticultural growth substrate.

9.2 Theoretical aim of the study

Theoretical aim of the study was to confirm that different *Sphagnum* moss species affect in different ways and that the modes of actions are species specific, negative, positive or neutral actions. The hypothesis that these modes of action are not only contributed to the *Sphagnum* mosses naturally low pH was confirmed. Only in the case of *R. sativus var. sativus* the added $CaCO_3$ had an increasing effect on seed germination, otherwise the added lime had a neutral effect on germination. It was also found in the light of literary review unlikely that the *Sphagnum* moss phenol compounds are responsible of the shown allelopathic effect. More studies of the *Sphagnum* mosses chemical composition are needed to reveal the true cause of allelopathic effect shown here.

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